JOURNAL OF DAIRY SCIENCE

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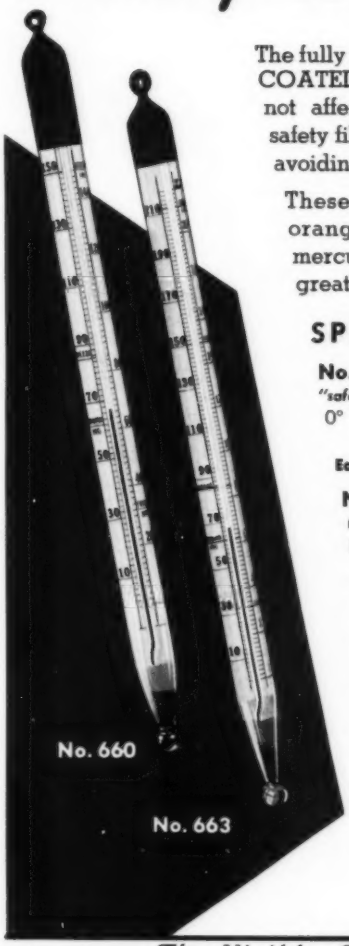

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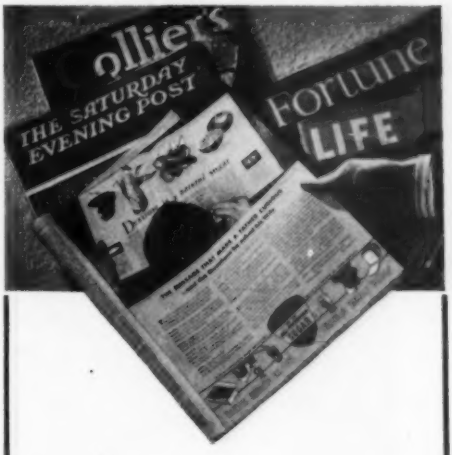

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JOURNAL OF DAIRY SCIENCE

VOLUME XXIV FEBRUARY, 1941 NUMBER 2

SOME FACTORS AFFECTING THE STABILITY OF CERTAIN MILK PROPERTIES. V. INTERRELATION OF CERTAIN METALS AND METALLIC IONS AND THE DEVELOPMENT OF OXIDIZED FLAVOR IN MILK[1]

O. F. GARRETT

New Jersey Agricultural Experiment Station, New Brunswick, N. J.

Contamination of milk with copper ions has long been recognized by researeh workers as one of the principal factors which causes the development of oxidized flavor. It seems to be generally believed that the action of copper is catalytic and it has been shown by Guthrie, Hand and Sharp (1) and by Hartman (2) that oxygen must be present in copper-contaminated milk for the oxidized flavor to develop. The speed of the oxidation reaction and the intensity of the oxidized flavor are, in part, dependent upon the concentration of copper ions.

Contamination of milk with iron is known also to cause the development of oxidized flavor. Brown, Thurston and Dustman (3) added ferric iron at the rate of 31, 62, and 93 parts per million parts of milk and observed that an oxidized flavor developed. The highest of these concentrations, however, did not give as an intense flavor as did 1.3 parts of copper per million parts of milk. Garrett and Hartman (4) observed that ferrous iron caused the development of oxidized flavor much faster than did an equivalent concentration of ferric iron. Greenbank's (5) observations were similar to those of Garrett and Hartman. Since an oxidation reaction is supposed to occur in the development of the oxidized flavor it would be expeeted that the more highly oxidized form of the iron would have the greater and more rapid effect but this is contrary to the above observations.

While studying the accelerative action of various metallic ions on the development of oxidized flavor in milk, it was observed that when copper and ferrous ions in equal molar concentrations were placed in milk the intensity of the oxidized flavor did not become as great as when either one of the ions was present alone. This is shown in table 1.

Received for publication August 22, 1940.

[1] Journal series paper of the New Jersey Agricultural Experiment Station, Department of Dairy Husbandry.

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TABLE 1

Effect of the addition of copper sulfate, ferrous sulfate and copper sulfate plus ferrous sulfate on the development of oxidized flavor in milk

	Intensity of oxidized flavor														
	.001 M*					.01 M*					.06 M*				
Hours	24	48	72	96	120	24	48	72	96	120	24	48	72	96	120
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
$CuSO_4$	1	1	1	2	2	1	1	1	2	2	1	3	4	4	5
$FeSO_4$	0	0	0	0	0	0	0	1	1	0	1	2	3	3	3
$CuSO_4$ + $FeSO_4$	0	0	0	0	0	0	0	0	0	0	0	1	1	2	3

* Concentration of the different metallic ions as moles per million parts of milk.

INTERRELATION OF COPPER AND MANGANESE

Divalent manganese is a lower oxidized form of the metal and, therefore can be oxidized to a higher valence. It was thought that, since cupric ions bring about the development of oxidized flavor in milk, divalent manganese might inhibit the oxidative action of copper. To test this deduction several samples of commercially pasteurized milk were contaminated with various molar concentrations of cupric and divalent manganese ions (in the form of $CuSO_4$ and $MnCl_2$). One of the samples of milk was either susceptible to oxidation or had been contaminated with enough copper in processing to cause the development of an oxidized flavor. The intensity of the oxidized flavor is expressed by numbers from 0 to 5. Doubtful cases are given a numerical value of 0.5. The results given in table 2 are averages obtained on five samples.

TABLE 2

Effect of cupric and divalent manganese ions on the development of oxidized flavor

Concentration* of metal		Intensity of oxidized flavor			
$CuSO_4$	$MnCl_2$	24 hrs.	48 hrs.	72 hrs.	96 hrs.
None	None	0	0.2	0.2	0.2
.01	None	0.5	1.3	2.2	3.0
.01	.01	0	0	0	0
.01	.02	0	0	0	0
None	.02	0	0	0	0
.06	None	1.0	2.4	4.0	5.0
.06	.06	0	0.2	0.6	1.0
.06	.12	0	0	0	0

* Expressed as moles per million parts of milk.

These results definitely show that divalent manganese ions inhibit the oxidative action of copper and completely prevent the development or greatly lessen the intensity of the oxidized flavor. The manganese did not impair the flavor of the milk in any way. Copper-contaminated milk to

which manganese was added had a slight metallic taste but even this seemed less intense than that found in the milk containing copper alone.

A greater variation in the concentration of the copper and manganese ions was made so that effective limits of the action of the manganese ions could be observed. The effects on the intensity of the oxidized flavor are shown in table 3 and in figure 1.

TABLE 3

Intensity of oxidized flavor in milk as influenced by wide variations in the concentration of copper and manganese

Concentration* of metal		Intensity of oxidized flavor			
$CuSO_4$	$MnCl_2$	24 hrs.	48 hrs.	72 hrs.	96 hrs.
None	None	0	0	1	2
.06	None	2	3	4	5
.06	.001	2	3	4	4
.06	.005	1	2	3	3
.06	.01	1	2	2	3
.06	.02	0.5	1	2	2
.06	.04	0	0.5	1	2
.06	.06	0	0	0	1
.01	None	1	2	2	3
.01	.0005	1	2	2	3
.01	.001	1	1	2	2
.01	.002	1	1	1	1
.01	.005	0.5	1	1	1
.01	.01	0	0	0.5	1
.01	.02	0	0	0	0
.01	.04	0	0	0	0
None	.01	0	0	0	0

* Expressed as moles per million parts of milk.

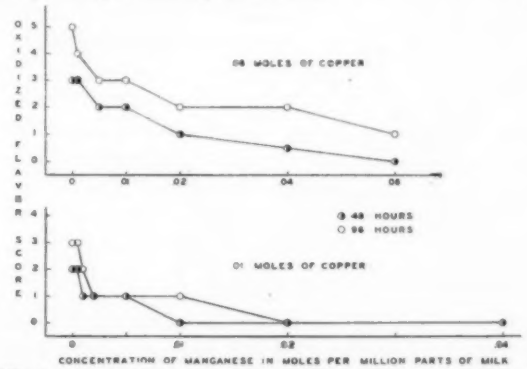
OXIDIZED FLAVOR SCORE
.06 MOLES OF COPPER
48 HOURS
96 HOURS
.01 MOLES OF COPPER
CONCENTRATION OF MANGANESE IN MOLES PER MILLION PARTS OF MILK

FIG. 1. Effect of concentration of divalent manganese on the development of copper-induced oxidized flavor.

The results shown in table 3 indicate that the concentration of manganese ions must be equal to or somewhat greater than the copper if the action of copper is to be completely inhibited, although a considerable retardation

takes place when the concentration of manganese is much lower than that of copper.

Since divalent manganese has such a strong inhibiting effect on the action of copper in developing the oxidized flavor, it might be assumed that the magnitude of the oxidation-reduction potential and the velocity of other oxidation reactions would be influenced. Accordingly, the oxidation-reduction potential and the concentration of ascorbic acid were measured on milks containing various amounts of copper and manganese. The results on oxidation-reduction potentials are shown in table 4 and those on ascorbic acid in table 5.

TABLE 4

Effect of various proportions of copper and manganese on the oxidation-reduction potential in milk

Concentration* of metal		Eh at 4° C.		
$CuSO_4$	$MnCl_2$	0 hrs.	24 hrs.	48 hrs.
None	None	.337	.323	.327
.01	None	.346	.357	.374
.01	.01	.350	.356	.374
.01	.02	.348	.357	.374
None	.02	.336	.323	.327
.06	None	.418	.380	.389
.06	.06	.419	.380	.389
.06	.12	.420	.380	.390

* Expressed as moles per million parts of milk.

TABLE 5

Effects of various proportions of copper and manganese on the ascorbic acid in milk

Concentration* of metal		Milligrams ascorbic acid per liter of milk		
$CuSO_4$	$MnCl_2$	0 hrs.	24 hrs.	48 hrs.
None	None	13.37	9.23	6.04
.01	None		0	0
.01	.01		0	0
.01	.02		0	0
None	.02		8.83	6.36
.06	None		0	0
.06	.06		0	0
.06	.12		0	0

* Expressed as moles per million parts of milk.

Contrary to expectations, the addition of divalent manganese had no effect on the magnitude of the oxidation-reduction potential or the rate of deterioration of ascorbic acid in uncontaminated or copper-contaminated milk. These results indicate that the mechanism of the oxidation of ascorbic acid is quite different from that responsible for the development of oxidized flavor. They also indicate that oxidized flavor does not necessarily accompany an increase in oxidation-reduction potential in milk.

The question arose as to whether the time at which manganese is added to milk relative to the copper is important and whether the development of oxidized flavor can be arrested once it has started. Manganese was added to milk before and after contamination with copper and the development of oxidized flavor was observed daily for 4 days. Copper was added at the rate of 0.02 moles and manganese at 0.06 moles per million parts of milk. The results are shown in table 6.

TABLE 6

Effect on the development of oxidized flavor in milk by adding manganese before and after contamination with copper

Time of adding manganese	Intensity of oxidized flavor			
	24 hrs.	48 hrs.	72 hrs.	96 hrs.
48 hrs. before Cu	0	0	0	0
24 hrs. before Cu	0	0	0	0
6 hrs. before Cu	0	0	0	0
1 hr. before Cu	0	0	0	0
Same time	0	0	0	0
1 hr. after Cu	0	0	0	0
24 hrs. after Cu	1*	1	1	0
48 hrs. after Cu	1	2*	2	2
72 hrs. after Cu	1	2	3*	2
Control plus Cu	1	2	3	4
Control	0	0	0	0

* Point at which manganese was added.

When the manganese was added to the milk at any time before or soon after contamination with copper a complete inhibiting effect on the development of the oxidized flavor was observed. An arresting effect on further development of the flavor was noted when the manganese was added 24, 48, and 72 hours after contamination with copper.

Strips of copper metal were immersed for 24 hours in milk which contained manganese and the milks were judged for oxidized flavor 24 hours after the copper was removed. In a similar manner pieces of manganese metal were immersed in milk which contained soluble copper and the intensity of the oxidized flavor was determined after 48 hours.

The results are given in tables 7 and 8.

TABLE 7

Effect on the development of oxidized flavor of immersing strips of copper metal in milk containing manganese

	Intensity of oxidized flavor after 48 hours
No Manganese	5
.06 M* $MnCl_2$	2
.12 M* $MnCl_2$	2
Manganese Metal	0
Control (no copper)	0

* Expressed as moles per million parts of milk.

TABLE 8

Effect on the development of oxidized flavor of immersing metallic manganese in milk containing copper

	Intensity of oxidized flavor after 48 hours
No Copper	0
.01 M* $CuSO_4$	1
.06 M* $CuSO_4$	1
.06 M* $CuSO_4$ (no Mn)	4
Control (No Cu, No Mn)	0

* Expressed as moles per million parts of milk.

Metallic manganese has an effect similar to that of divalent manganese in retarding the development of oxidized flavor in copper-contaminated milk.

INTERRELATION OF IRON AND MANGANESE

Divalent manganese was added to milks containing ferrous iron with results on oxidized flavor very similar to those obtained with manganese and copper. The data are given in table 9.

TABLE 9

Effect of ferrous and divalent manganese ions on the development of oxidized flavor

Concentration* of metal		Intensity of oxidized flavor		
$FeSO_4$	$MnCl_2$	24 hrs.	48 hrs.	72 hrs.
None	None	0	0	0
.01	None	1	1	2
.01	.01	0	0	0
.01	.02	0	0	0
None	.02	0	0	0
.06	None	1	2	4
.06	.06	0	0	1
.06	.12	0	0	0

* Expressed as moles per million parts of milk.

Aluminum chloride was substituted for manganese chloride in the experiments with copper but no retarding effect on the development of oxidized flavor was observed.

Practical application of the findings of these experiments has not been attempted.

It remains to be proved that manganese will inhibit the development of oxidized flavor in milk not contaminated with copper or iron but susceptible to oxidation.

SUMMARY

The results of these studies indicate that:

Divalent manganese added to milk contaminated with copper or iron completely inhibits or greatly retards the development of oxidized flavor.

Metallic manganese exhibits an effect similar to that of divalent manganese.

Divalent manganese has no effect on the magnitude of the oxidation-reduction potential or the rate of deterioration of ascorbic acid in uncontaminated or copper-contaminated milk.

Aluminum does not prevent the development of oxidized flavor in copper-contaminated milk.

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THE TIME OF OVULATION IN CATTLE*

J. E. BREWSTER AND C. L. COLE
Michigan State College, East Lansing, Mich.

The increased use of artificial insemination in cattle breeding has put added importance on the optimum time for mating to obtain the highest possible breeding efficiency.

It is known that the life of the spermatozoa is relatively short in the female reproductive tract; consequently, the time of ovulation is of considerable importance if maximum breeding efficiency is to be obtained.

The work reported here was conducted with the purpose of determining as nearly as possible at what stage of the estrual cycle ovulation occurs. Information was sought concerning the difference in time of ovulation between the types and breeds of cattle, the influence of age upon the time of ovulation, which ovary functioned more often, and the time of day ovulation was most likely to occur. No attempt was made to determine the length of estrus.

The results reported in the literature on the time of ovulation in cattle have been variable and the number of animals used has been small. Hammond (6), reporting his own work and that of others, concludes that ovulation occurs spontaneously in the cow from 24 to 48 hours after the onset of estrus. In a later publication, Hammond (7) states that ovulation occurs 27 hours after the onset or just after the end of estrus.

Beschlebnov (3) examined 5 cows 22 to 30 hours after mating and ovulation had occurred in all of them. In 4 others, ripe follicles were found 21 to 26 hours after mating. Nine cows examined 34 to 49 hours after breeding had all ovulated a considerable time before examination.

Andreev (2) states ovulation occurs 30 to 40 hours after the onset of estrus.

Kirillov (8) divided his herd into two parts. One part he mated at the beginning of estrus, the other, after 18 to 24 hours. The majority of cases indicated that ovulation occurred after 18 to 24 hours because only 26 per cent of these cows had to be rebred while 60 per cent of those bred at the beginning of estrus returned.

Cole (5) states that ovulation occurs one day postestrum.

Miller, Swett, Hartman, and Lewis (9) recovered a fertilized egg from the uterus of a cow 48 hours after the appearance of estrus in the cow. The second cow that they slaughtered had not ovulated. An ovum was recovered from a third cow, but it was not fertilized although she had been bred.

Murphy, McNutt, Zupp, and Aitken (10) report examining a cow per rectum that ovulated between 40 and 64 hours after the beginning of estrus.

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Werner, Casida, and Rupel (13) after examining 15 heifers at half-day intervals, concluded that ovulation occurred within the first day after cessation of estrus.

Nalbandov and Casida (11) from examinations at 15 estrual periods, determined that time of ovulation varied from $2\frac{1}{2}$ to $22\frac{3}{4}$ hours after the end of estrus. The average time of ovulation was $11\frac{3}{4}$ hours postestrus. Their observations indicate that estrus begins more often in the forenoon and that ovulation takes place more often in the forenoon. In 11 out of 17 cases ovulation occurred between 4:00 and 10:30 A.M.

Reese and Turner (12) in studies at the Missouri Experiment Station came to the conclusion that the right ovary is functionally more active than the left. This difference accounts for the greater number of pregnancies in the right uterine horn than in the left horn.

Hartmann (1) quotes Kupfer as stating that in 24 cows he found a larger number of corpora lutea of all ages in the right ovary, while in 12 cows the larger number was in the left. He also quotes from Atkeson's work (unpublished) at the Idaho Experiment Station that there was an 80 per cent advantage for the right ovary, with 166 ovulations from the right ovary and 92 from the left.

Clark (4) in a study of 704 cases of single pregnancies concludes that both ovaries function with approximately equal frequency.

EXPERIMENTAL

This study was conducted from June, 1939, to April, 1940. The animals used were females of various ages from the dairy and beef cattle herds at Michigan State College.

Each cow in estrus was confined in the barn and tried with a steer at two-hour intervals until she refused to stand for the steer to mount. The time of ovulation was determined by palpating the ovaries through the rectum. Both of the ovaries were palpated at each examination, beginning a few hours after the cow went off estrus. Examinations were made of the ovaries at two-hour intervals until she had ovulated. If the estrual period was unusually long, observations were made before she went off heat. The time for going off estrus and the time of ovulation were computed by taking an average between the last two observations in each case. Nine of the cows examined were slaughtered to verify the observations made by ovarian palpation.

It was not always possible to determine the end of estrus or the time of ovulation within the two-hour limit. Results obtained in these cases were not used in computing averages.

Seventy-three examinations were made on 47 cows. Seventy ovulations were recorded and in 3 cases ovulation did not occur. Fifty-three of the 70 ovulations are included in computing averages. The remaining 17 were not checked within the two-hour interval.

Of the ovulations reported, 5 were from dairy cows, 13 from Shorthorns, 15 from Angus, and 20 from Herefords.

Several follicles erupted while they were being palpated. These cases raised the question as to the effect of palpation on the time of ovulation. All of these instances occurred at about the time ovulation was expected. Since a comparable amount of pressure exerted on the follicle at other times failed to cause it to rupture, it was concluded that any error in the time of ovulation caused by palpation would fall within the 2-hour limits.

Ovulation was found, in all except three instances, to occur within the first day after the end of estrus. One cow, an Angus, ovulated on two occasions before she went off estrus. Another Angus ovulated 26 hours after the end of estrus. The average time of ovulation was 13.57 ± 0.68 hours from the end of estrus. Eighty-eight and seven-tenths per cent of the ovulations occurred between 6 and 19 hours, and 67 per cent occurred between 8 and 16 hours after the end of estrus.

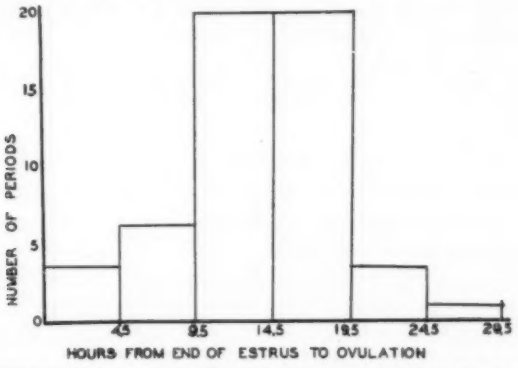

FIG. 1. Histogram showing time of ovulation in cattle after the end of estrus.

Exact records were not kept as to the beginning of estrus; however, a great deal of variation was noted in the length of the estrual periods. Some cows stayed in estrus about 12 hours while others were in heat as long as 36 hours.

The mean time of ovulation for dairy cows was 15 ± 2.6 hours; Shorthorn, 12.69 ± 1.15 hours; Angus, 13.93 ± 1.68 hours; and Hereford, $13.5 \pm .87$ hours postestrus. These averages are so close that they probably do not indicate any variation between breeds or types. The greatest difference was 2.31 hours between dairy and Shorthorn cows. In each case the numbers were too small to be significant.

In this study successive ovulations occurred from the same ovary in some instances and in others successive ovulations occurred from alternate ovaries. Of the 70 ovulations recorded, 35 were from the left ovary and 35 from the right ovary. Of the 53 ovulations used in computing averages,

26 were from the left ovary and 27 from the right ovary. The average time of ovulation from the left ovary was 13.19 ± 1.14 hours, while from the right ovary the average was 13.93 ± 0.75 hours.

One cow, a Jersey, had a twin ovulation. She ovulated from the left ovary 11 hours after the end of estrus and from the right ovary 16 hours after the end of estrus. The two ovulations were checked on slaughter.

The greatest difference was in the time of ovulation between cows that had calved and heifers that had not calved. There were 16 ovulations recorded among yearling and two-year-old heifers. They ovulated an average of 11.4 ± 0.99 hours after the end of estrus, while 37 cows from 3 to 12 years of age ovulated $14.48 \pm .84$ hours after estrus, a difference of 3.04 hours. This difference was found to be significant by the use of Fisher's test of significance between means of small samples. Difference was found to be 2.12 while 2.08 was required to show a significant difference.

Ovulation occurred at almost every hour of the day. Of the 53 ovulations reported, 22 were between 12:00 o'clock P.M. and 12:00 o'clock A.M., while 31 were between 12:00 o'clock A.M. and 12:00 o'clock P.M.

Nine cows were slaughtered after ovulation had occurred as determined by ovarian palpation. In each case, post-slaughter examination confirmed these observations.

SUMMARY

1. Observations were made at 73 estrual periods of 47 cows of beef and dairy breeds. The time from the end of estrus to ovulation was determined by rectal palpations of the ovaries.

2. Ovulation occurred within the first day after the end of estrus, the average time from the end of estrus to ovulation for 53 ovulations being 13.57 ± 0.68 hours. Sixty-seven per cent of the ovulations occurred between 8 and 16 hours after the end of estrus.

3. Time of day and breed or type of animal had no influence on the time of ovulation.

4. Both ovaries produced follicles with equal frequency.

5. Heifers required an average of 3.04 hours less time to ovulate than cows that had calved.

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AVENIZED VERSUS STANDARD PARCHMENT FOR WRAPPING PRINT BUTTER*

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The sizing of parchment and other packaging materials with oat flour (Avenex) for the purpose of retarding oxidative deterioration of fatty foods was developed under patents assigned to the Musher Foundation, Inc. Koenig (1), Dahle and Josephson (2), and Corbett and Tracy (3) have reported favorably on the use of Avenized parchment for wrapping print butter. The purpose of the work herein reported was to secure further data on the value of Avenized parchment in retarding the surface deterioration of print butter.

PLAN OF EXPERIMENT

Through the cooperation of a local butter firm, the butter used was selected from the supply of regular shippers. The desired number of one-pound prints of butter from each churning was taken direct from the printing machine and wrapped by hand. The parchment wrappers used were manufactured by the Kalamazoo Vegetable Parchment Company and by the Patterson Parchment Paper Company. Two shipments of parchment were secured from each manufacturer, part in each shipment being standard thirty-pound parchment and part standard parchment treated with oat flour at the factory. All of the butter prints from any one churning were wrapped in parchment supplied by the same manufacturer, some in the standard parchment and the others in the treated parchment.

The work involved two groups of trials, one in which the butter was scored by local graders, and the other in which the aid of graders in other sections of the country was enlisted.

For the first group of trials, butter from forty churnings was placed in storage in June, 1938, from forty churnings in September, 1938, and from thirty churnings in March, 1939. In each instance one-half was neutralized sour cream butter that had been graded 90, and one-half sweet cream butter that had been graded 93. Seven one-pound prints of butter were taken from each churning. One print was graded within 24 hours after printing. Sufficient prints were prepared so that a separate pound print was available for

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grading at the storage intervals selected. The wrapped prints were placed in standard corrugated boxes and set in storage at 40° F.

The June butter was held in storage for fifteen and thirty days, and the samples selected in September and March for fifteen, thirty and forty-five days. At the end of each storage period one print from each churning wrapped in the two different parchments was removed for scoring. Each print of butter was so labeled that those grading it could not know the type of parchment used for wrapping. The samples were not identified as pairs. It has been the experience of the authors that, if the difference in flavor between two samples of butter is slight, the necessity of giving divergent scores may magnify the actual difference. If the use of Avenized parchment retards the flavor deterioration of the butter, the fact should be disclosed by routine scoring of random, not-paired samples. The grading was done by Federal graders and the senior authors working together in groups of two or more. In grading the butter, attention was given only to the surface of the butter. Thin layers of the surface were removed by the graders, using flat hard wood sticks or metal spatulas.

To secure the judgment of men from other sections of the country, in August, 1939, butter was selected from twenty-five churnings which had been scored 93, and after storage different lots were scored by judges located in Minnesota, Illinois and Pennsylvania. This was repeated in November, 1939, with butter from the same number of churnings which had been scored 90 and 91.

The fat aldehyde value of Schibsted (4) was determined for the butterfat from the September, 1938, churnings and from the August, 1939, and November, 1939, churnings which were scored at the University of Minnesota. The butterfat used for these determinations was from the outer one-quarter inch layer of butter cut from each side and end of the print. The fat aldehyde test was made in a darkened room as this procedure was shown necessary in preliminary experiments. Barnicoat and Palmer (5) found the fat aldehyde test to be of some value in studying minor oxidative changes in butter. The peroxide test (Lea 6) was considered by them to be of little use.

RESULTS OF EXPERIMENTS

Group I—Butter Scored by Local Graders. A comparison is presented in table 1 of the number of times in which the prints from the same churning wrapped in the two types of parchment were scored the same, and in which the print wrapped in one parchment was scored higher than the print wrapped in the other parchment at the various periods of storage. Over 81 per cent of the samples wrapped in the two types of parchment were scored the same at the end of fifteen days, over 85 per cent were graded the same at the end of thirty days and 90 per cent at the end of forty-five days. Since most print butter reaches the consumer within thirty days, it may be proper

TABLE 1

Comparison of scores of print butter wrapped in standard and Avenized parchment after storage for 15, 30 and 45 days

Score of fresh butter	Days in storage	Total comparisons	Avenized and standard parchment wrapped prints scored same	Avenized parchment wrapped prints scored higher	Standard parchment wrapped prints scored higher
		No. churnings	*No. churnings*	*No. churnings*	*No. churnings*
93	15	55	39	7	9
	30	55	44	4	7
	45	35	29	0	6
90	15	55	51	1	3
	30	55	50	3	2
	45	35	34	1	0

to give more attention to the data for this period. Out of 110 comparisons, 94 were given the same grade at the end of thirty days indicating that the graders could detect no difference in these samples, half of which were wrapped in Avenized parchment. Of these 94 comparisons, 44 represented butters which originally were scored 93 and fifty were graded 90 when fresh.

TABLE 2

Samples of 93 score butter stored for various periods and in different wrappings distributed according to loss in score

Loss in score	After 15 days storage		After 30 days storage		After 45 days storage	
	Standard parchment	Avenized parchment	Standard parchment	Avenized parchment	Standard parchment	Avenized parchment
	No. samples	*No. samples*	*No. samples*	*No. samples*	*No. samples*	*No. samples*
0 –0.50	45	45	16	15	5	2
0.51–1.00	10	10	25	25	15	16
1.01–1.50	.....	.....	4	4	4	6
1.51–2.00	.....	.....	7	8	6	6
2.01–2.50	.....	.....	.....	.....	2	1
2.51–3.00	.....	.....	2	3	2	3
Over 3.00	.....	.....	1	.....	1	1
Total	55	55	55	55	35	35

The samples are distributed in tables 2 and 3 according to the amount of loss in score. These frequency distributions show a striking similarity in the losses in score of the prints wrapped in the two types of parchment. The decrease in score of the butter with age is clearly shown but this does not appear to have been influenced by the type of parchment.

The samples are distributed in tables 4 and 5 according to the aldehyde values of the butterfat. These data support the conclusion drawn from the flavor scores that the wrapping of the butter in Avenized parchment rather

TABLE 3

Samples of 90 score butter stored for various periods and in different wrappings distributed according to loss in score

Loss in score	After 15 days storage		After 30 days storage		After 45 days storage	
	Standard parchment	Avenized parchment	Standard parchment	Avenized parchment	Standard parchment	Avenized parchment
	No. samples	*No. samples*	*No. samples*	*No. samples*	*No. samples*	*No. samples*
0 –0.50	48	48	31	31	4	5
0.51–1.00	2	3	18	18	10	9
1.01–1.50	.....	0	.....	.....	1	1
1.51–2.00	2	1	3	3	11	10
2.01–2.50	.....	0	.....	.....	1	1
2.51–3.00	2	2	.....	.....	3	3
Over 3.00	1	1	3	3	5	6
Total	55	55	55	55	35	35

TABLE 4

Samples of 93 score butter stored for various periods and in different wrappings distributed according to the aldehyde value of the butterfat

Aldehyde value	After 15 days storage		After 30 days storage		After 45 days storage	
	Standard parchment	Avenized parchment	Standard parchment	Avenized parchment	Standard parchment	Avenized parchment
	No. samples	*No. samples*	*No. samples*	*No. samples*	*No. samples*	*No. samples*
Under .20	19	15	14	13	12	11
.20–.39	.....	2	2	1	1	1
.40–.59	1	2	2	1	1	1
.60–.79	.....	.....	.....	2	2	.....
.80–.99	.....	1	2	1	1	.....
1.00 and over	.....	.....	.....	2	3	7
Mean	0.12	0.20	0.23	0.34	0.40	0.62

TABLE 5

Samples of 90 score butter stored for various periods and in different wrappings distributed according to the aldehyde value of the butterfat

Aldehyde value	After 15 days storage		After 30 days storage		After 45 days storage	
	Standard parchment*	Avenized parchment	Standard parchment	Avenized parchment	Standard parchment	Avenized parchment
	No. samples	*No. samples*	*No. samples*	*No. samples*	*No. samples*	*No. samples*
Under .20	9	10	7	2	1	.....
.20–.39	1	.....	1	5	2	.....
.40–.59	4	2	2	2	2	2
.60–.79	5	5	4	2	4	3
.80–.99	.....	1	1	2	3	4
1.00 and over	.....	2	5	7	8	11
Mean	0.36	0.37	0.67	0.88	0.97	1.26

* 1 sample lost.

than standard parchment did not retard oxidative deterioration of the butter used in these experiments.

That the aldehyde value is at least indicative of the degree of fat deterioration is shown by the increases in the aldehyde values of the samples with storage, and by the higher aldehyde values of the fat from the originally 90-score butter.

Group II—Butter Scored by Local and Out-of-State Graders. On August 28, 1939, eight one-pound prints were secured from each of twenty-five different churnings of 93-score salted butter. Four prints from each churning were wrapped in standard parchment and four prints in Avenized parchment. The butter was packed in four standard fiber butter boxes, one print wrapped in standard and one print wrapped in treated parchment from each churning in each box. The replicate prints occupied the same position in each box. The butter was held in storage at −10° F. until September 25, when it was placed at 40° F. On October 3, 1939, the fiber boxes containing the butter were placed in insulated boxes with fifteen pounds of dry ice in each box. These boxes were shipped by express, one to Professor C. D. Dahle, Pennsylvania State College, one to N. W. Hepburn, American Butter Institute, Chicago, Illinois, and the other to Professor P. H. Tracy, University of Illinois. The remaining box was held at room temperature at the University of Minnesota for 36 hours and then placed at 40° F. until October 9 when it was scored. Thus the butter was stored for 4 weeks at −10° F. and for 2 weeks at 40° F. The butter shipped to Mr. Hepburn was scored by a Federal grader, by a Chicago Mercantile Exchange grader and by a commercial grader. Professors Dahle and Tracy and their associates cooperated in scoring the butter sent to their respective institutions. All graders were instructed to grade the surface of the butter.

As shown in table 6, the mean of the scores given by grader No. 2 for the

TABLE 6

Mean scores by different graders of originally 93 score butter after storage at −10° F. for 4 weeks and at 40° F. for 2 weeks

Parchment	Grader					
	1	2	3	4	5	6
Standard	91.70	90.56	90.22	89.62	90.06	87.29
Avenized	91.64	91.28	90.37	89.75	90.10	87.34
Difference	0.06	0.72	0.15	0.13	0.04	0.05

samples wrapped in Avenized parchment was 0.72 higher than the mean of the scores given by this grader for the duplicate samples wrapped in standard parchment. This difference is statistically significant as determined by Fischer's (7) t. test. Four of the other five graders also gave a slight advantage in average score to the butters wrapped in the Avenized parchment.

These differences in scores, however, are not statistically significant. Grader No. 2 scored sixteen of the twenty-five samples wrapped in Avenized parchment higher than the duplicate samples wrapped in standard parchment, six the same and only three samples wrapped in the standard parchment higher than the samples wrapped in the Avenized parchment. Taking the composite scores of the other graders, in 46 instances the Avenized parchment wrapped sample was scored higher, in 38 the standard parchment wrapped sample and in 39[1] they were scored the same. Considering the scores of all graders on the butter from each individual churning, the average difference in score between the samples from the same churning wrapped in the different parchments varied no more than 0.25 point in 14 cases. The prints wrapped in the standard parchment had an average score of from 0.26 to 0.75 points higher in 4 instances and the prints wrapped in Avenized parchment a like advantage in average score an equal number of times. Prints from two churnings which were wrapped in the Avenized parchment received an average score of 1.167 and 1.083 points, respectively, higher than prints from the same churning which were wrapped in the standard parchment. The differences in scores of these churnings and of one other in which the difference was 0.667 in favor of the butter wrapped in the Avenized parchment approach statistical significance according to Fischer's (7) t. test.

There was no appreciable difference in the aldehyde values of the butterfat from the two sets of samples. The mean aldehyde values were 0.088 and 0.079 for the butterfat from the prints wrapped in standard parchment and Avenized parchment, respectively. The aldehyde value for the butterfat from only four of the Avenized parchment wrapped prints was above 0.10. The butterfat from the same churnings plus one other churning of the prints wrapped in standard parchment had aldehyde values above 0.10.

Despite the low scores given the butter by certain of the graders, the aldehyde values show that very little oxidation had occurred. Schibsted (4) reported an aldehyde value of 0.50 for butter oil described as good by organoleptic test. Barnicoat and Palmer (5) considered that the period of induction in fat oxidation appears to end at about 20 units of fat aldehyde value. They obtained values in most cases less than 0.50 unit in testing a considerable number of butter samples. As previously noted, the aldehyde values of the butterfat from the originally 90 score neutralized-sour cream butter were higher than those from the butterfat from the high quality sweet cream butter used in these experiments. Since an antioxidant should be most effective in retarding oxidation in a product in which oxidation would otherwise progress appreciably, another trial was made using originally 90 and 91 score neutralized-sour cream butter. The procedure was

[1] Two of these graders did not score one sample, which accounts for there being only 123 instead of 125 comparisons.

the same as in the preceding trial except that the butter was stored for 33 days at 40° F. beginning on December 20, 1939. The butter was scored after storage by the same graders or groups of graders plus one other, a Federal grader stationed in Minnesota.

The mean scores of the various graders for the prints wrapped in the two types of parchment are shown in table 7. As in the previous trial with

TABLE 7

Mean scores by different graders of originally 90 and 91 score butter after storage for 30 days at 40° F.

Parchment	Grader							Average
	1	2	3	4	5	6	7	
Standard	88.48	89.18	90.32	89.32	87.34	87.48	88.34	88.65
Avenized	88.64	89.68	90.52	89.70	88.26	88.04	88.22	89.01
Difference	0.18	0.50	0.20	0.38	0.92	0.56	0.12	0.36

originally high quality sweet cream butter, the conclusion that would be reached would depend upon which grader scored the butter. Grader No. 5 scored 17 of the prints wrapped in the Avenized parchment higher, 4 lower, and 4 the same as the prints from the same churnings wrapped in the standard parchment. Grader No. 7 reversed this decision and scored only 5 of the prints wrapped in the Avenized parchment higher, 10 lower, and 10 the same as the prints from the same churnings wrapped in the standard parchment. Six of the seven graders, however, gave an advantage in average score to the butter wrapped in the Avenized parchment. The differences in score are statistically significant in the case of the scores given by graders two, four, five and six. Thus, if the scores of these four judges were taken, the conclusion would be that the use of Avenized parchment was of definite value in retarding deterioration of the butter, but from the scores of the three other judges, that the use of Avenized parchment was of no value. The average difference of 0.36 in the scores of all judges in favor of the butter wrapped in the Avenized parchment is significant. Considering the scores of all judges on the butter from each individual churning, the average difference in score of the prints wrapped in the two types of parchment was no greater than 0.25 in 10 churnings. The butter from only one churning wrapped in the standard parchment was given an average advantage in score of more than 0.25, whereas in 9 churnings the butter wrapped in Avenized parchment was given an average advantage in score of 0.26 to 0.75, and in 5 churnings an advantage of from 0.76 to a maximum of 1.07. In only three of these churnings, however, was the difference sufficiently great to be statistically significant.

As shown in table 8 there was a marked increase in the aldehyde value of the butterfat during storage although this appears not to have been influenced by the parchment paper used.

TABLE 8

Samples of 90 and 91 score butter stored at 40° F. for 33 days in different wrappings distributed according to the aldehyde value of the butterfat

Aldehyde value	Original butter	After 33 days storage at 40° F. wrapped in	
		Standard parchment	Avenized parchment
	No. samples	*No. samples*	*No. samples*
0 –0.50	12	9	9
0.51–1.00	7	2	1
1.01–1.50	5	3	1
1.51–2.00	1	2	2
2.01–2.50	....	5	7
Over 2.50	....	4	5
Mean	0.55	1.32	1.57

DISCUSSION

Fallibility of the human judgment of taste and odor is a limiting factor in any work involving the score of butter. Differences in the scores placed on a sample by two or more graders or groups of graders may be due to a difference in the standards of the graders. If this is the only divergent factor, the ranking of the samples should be the same. The difference between the highest and lowest average scores given by two graders on any group of 25 samples was 4.41 on the originally 93 score butter and 2.98 on the originally 90 and 91 score butter. More than a difference in standards is represented here, however, as the ranking of the samples within a group by the different graders was widely different. To some degree these variations may be due to differences in the treatment to which the several lots of butter were subjected after they left University Farm, St. Paul. This seems to be of negligible importance, however, as three of the graders (Nos. 3, 4 and 5) scored the same samples of butter. Their scores are not less variable than those of the other graders. It is evident that conclusions based on the scoring of butter where the differences are small may be erroneous if based on the scores of only one grader or group of graders. If the use of Avenized parchment actually retards deterioration of butter, the use of paired samples might have shown a more consistent difference in favor of the samples wrapped in that parchment.

CONCLUSIONS

1. The use of Avenized rather than standard parchment paper as a wrapper for print butter has a very slight effect in retarding deterioration of the flavor of the surface of such butter.

2. Retardation of loss in score is more likely to result from the use of Avenized parchment with neutralized sour cream butter than with sweet cream butter.

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BODY SIZE AND MILK PRODUCTION

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BODY SIZE AND PRODUCTIVE CAPACITY

The senior author has suggested (1) that in general the total efficiency of energy utilization by animals $\frac{\text{energy in the product (milk, eggs, etc.)}}{\text{energy in the food}}$ is independent of body size. Involved in this theory is the assumption that an animal's capacity to take in food per day is, as a rule, a certain multiple of its maintenance requirement or also its rate of fasting katabolism. This assumption was supported by some data on the food capacity of baby chicks, rabbits, sheep, swine, and steers, which indicated that the rate of intake of total food energy in these animals was four to six times as great as their rate of fasting katabolism. According to this theory the capacity for rate of production (energy in meat, milk, or eggs per unit of time) of large and small animals should be proportional to their respective rate of fasting katabolism; or, since this metabolic rate is proportional to the ¾ power of body weight (2), the capacity for rate of production should also be proportional to the ¾ power of body weight. Thus the rate of lactation per unit of metabolic body size ($kg.^{¾}$)* should be the same for large and small animals.

This deduction was supported by Eckles's data (3) on average milk production and body weight of Jersey and Holstein cows. The energy in the yearly milk production (calculated according to Möllgaard (4)) of the Jerseys was 25.1, that of the Holsteins 23.9 thousand kilocalories per $kg.^{¾}$, a difference of only 5 per cent, whereas it was 5.65 and 4.87 thousand kilocalories respectively per kg. of body weight, a difference of 16 per cent. If the milk energy is calculated according to Overman and Gaines (5), then the difference in milk energy per unit of the ¾ power of body weight is only 2 per cent; that per kg. body weight, 11 per cent.

Our theory that capacity for rate of milk production is closely related to the ¾ power of body weight has been rather well confirmed by Brody and his coworkers in comparing Jersey with Holstein cows (6, 7), goats with cows (8), and rats with cows (9). Rats as well as high-producing cows gave off about twice as much energy in their daily milk as they would have lost daily as heat during fasting.

GAINES' THEORY OF BODY SIZE AND MILK PRODUCTION

In contradiction to our ¾ power rule, W. L. Gaines (10) deduced in a

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* Since the metabolic body size is the body weight in kg. raised to the ¾ power, the unit of this size is $kg.^{¾}$.

recent article from 14,000 milk records obtained by Kleinerg in Czechoslovakia that "the results effectively dispose of the notion that . . . 'metabolic body size' . . . is proportional to the ¾ power of live weight in the sense that the ¾ power of live weight represents the *limit* of potential capacity in cows to do sustained work in lactation" and ". . . the results lend support to the notion that as between cows of different live weights, potential lactation capacity (present or capable of development) is proportional to live weight."

Since the rate of fasting katabolism is approximately proportional to the ¾ power of live weight, the idea that productive capacity is proportional to live weight itself, (that is, its first power) leads to a complication in the formulation of energy utilization.

A part, M, of the total food energy, U, is used up for maintenance. From the food energy available for production $(U - M)$, only a part (net energy) appears actually as the energy of the animals' product, A, so that

$$A = e_p\ (U - M) \qquad 1)$$

where e_p is the partial efficiency (food value) for production.

The energy M of the food for maintenance is greater than the energy B lost from the body during fasting, so that

$$B = e_m\ M, \text{ or} \qquad M = \frac{B}{e_m} \qquad 2)$$

where the fraction e_m stands for the partial efficiency (food value) for maintenance.

By combining equations 1 and 2 one obtains the following result:

$$A = e_p\ U - \frac{e_p}{e_m} B \qquad 3)$$

Equation 3 can be generally valid only if dimensionally correct. This means that the terms (A), $(e_p\ U)$ and $(\frac{e_p}{e_m} - B)$ must be of the same dimension. One term cannot, for example, be proportional to body surface if the other is proportional to body weight since one cannot add weights and surfaces; or if one term is proportional to a certain power function of body weight, the two others must be proportional to the same power function of body weight. If the partial efficiencies e_p and e_m are independent of body size (the simplest and, to our present knowledge, the most reasonable assumption in line with experience), then U (daily food energy) and A (daily energy in the animals' product such as milk) must be proportional to the same power function of body weight as the daily fasting katabolism, B.

For a great variety of warm-blooded animals the fasting katabolism may be predicted by the equation

$$B = 72\ W^{¾} \text{ kilocalories per day}$$

where W stands for the body weight in kilograms (2).

If this relation of body weight and fasting katabolism is valid for dairy cows, then their daily food energy (U) and the energy in their daily production (A) should be proportional to the ¾ power of their body weight.

If, on the other hand, according to Gaines' theory, the rate of production, A, were proportional to body weight, one would have to make one of two assumptions: either (1) the rate of food intake, U, would also have to be proportional to body weight and the efficiency of food utilization for maintenance, e_m inversely proportional to the 4th root of body weight; or (2) with the rate of food intake proportional to the ¾ power of body weight, the efficiency of food utilization for production e_p would have to be proportional to the 4th root of body weight. It is hard to find a theoretical or an empirical justification for such complicating assumptions that food values (partial efficiency of food utilization) for maintenance or for production should be functions of body weight.

Theoretical complications alone should, of course, not prevent the acceptance of Gaines's idea that the productive capacity of dairy cows is proportional to body weight, provided the theory carries enough empirical evidence. The evidence, supplied by Gaines, however, is open to criticism.

The exponents for body weight, c, in Gaines' table 1 vary from 0.104 to 2.961; and an average calculated from these figures does not at first sight seem convincing enough to "effectively dispose" of an otherwise rather well-supported theory. In using these tremendously variable figures, Gaines has given the same statistical weight to 653 results on "Berner" as to 8367 results on "Simmentaler" cows. If the results in his table 1 are weighted according to the number of observations, then the rate of milk production is on the average proportional to the 0.837th rather than the 1.021st power of body weight. If thus, the Czechoslovakian data used by Gaines are evaluated according to their statistical weight, then they support the ¾ power rule of productive capacity better than they support Gaines' own theory.

In a later paper Gaines (11), no longer used the method of least squares as applied to the data from Czechoslovakia. Having given the milk yield per unit body weight of 32 Holstein cows from a record of the United States Department of Agriculture, he summarizes the results by the equation:

$$\frac{FCM}{W} = 15.11 - 0.00304\ W$$

where FCM = pounds milk corrected to 4 per cent fat content.
W = body weight in pounds.

On the basis of this equation one can demonstrate that these data fit the ¾ power rule of productive capacity better than they fit Gaines' theory. For a change in weight from 1000 to 2000 pounds the FCM/W decreases 25 per cent whereas the $FCM/W^{¾}$ would decrease only 11 per cent.

One may calculate from the equation above that for the 32 records the FCM is most nearly proportional to the 0.58th power of body weight or nearly to the body surface.

The calculation may be carried out as follows:

$$\frac{FCM}{W} W^X = (15.11 - 0.00304\ W) W^X$$

This expression should be constant, so that

$$(15.11 - 0.00304\ W_1)\ W_1{}^X = (15.11 - 0.00304\ W_2)\ W_2{}^X$$

or

$$\left(\frac{W_2}{W_1}\right)^X = \frac{15.11 - 0.00304\ W_1}{15.11 - 0.00304\ W_2}$$

Thus

$$X \log \frac{W_2}{W_1} = \log \frac{15.11 - 0.00304\ W_1}{15.11 - 0.00304\ W_2}$$

and for $W_1 = 1000$ and $W_2 = 2000$:

$$X = \frac{\log 1.337}{\log 2.000} = 0.42$$

consequently

$$FCM \frac{W^{0.42}}{W} \text{ or } \frac{FCM}{W^{0.58}} = k$$

If the method of least squares were applied to the logarithms of body weight and milk yield of the 32 Holstein cows, a regression coefficient (representing the best fitting exponent of body weight) close to 0.6 would probably result.

BODY SIZE AND MILK PRODUCTION IN THE UNIVERSITY OF CALIFORNIA HERD

To get information on the relation of body size and milk production under well-defined conditions we analyzed data collected at this Station during fifteen years on milk production and body weight and height at withers of first-lactation Jersey and Holstein cows milked thrice daily on semi-official test. The feed and management has been uniformly the same for all the 42 Jerseys and 24 Holsteins whose records have been used in this study. The means of these data are summarized in table 1.

TABLE 1

Body size and rate of milk production of 2-year-old cows, first lactation, three milkings per day

Cows	Mean body weight		Mean height at withers	Mean milk energy in 10 months in million kilocalories	Relative lactation level. Mean milk energy per day per kg.$^{\frac{3}{4}}$
	lbs.	*kg.*	*cm.*		*kilocal.*
42 Jerseys	928	421	125	3.91	139.0
24 Holsteins	1203	546	134	4.30	125.5

If the method of least squares is applied to a linear interpolation of the logarithms of the milk-energy yields in 10 months and the logarithms of body weight, then regression coefficients of -0.01 within the Jerseys and -0.06 within the Holsteins result; and these indicate that the yield of milk energy per cow in this herd tends to be larger for the lighter animals within each breed. This finding agrees with earlier observations by Indermühle (12) on Simmentaler cows, which led to the suggestion (1) that, notwithstanding the validity of the ¾-power law of productive capacity in gen-

eral, an optimal body size for most efficient milk production might exist within one breed.

If the logarithm of 10 months' milk energy and of body weight of the 24 Holsteins and 42 Jerseys together are subjected to the method of least squares for linear interpolation, then a regression coefficient of 0.277 results for these 66 cows, leading to the conclusion that rate of production in these 66 cows is nearly proportional to the cube root of body weight.

A similar calculation with the height at withers instead of body weight as the characteristic of size leads to a regression coefficient of 1.033 between the logarithms of yield in milk energy and height. Thus in these 66 cows the rate of production appears to be directly proportional to height. Since in similar bodies height, as a linear dimension, is proportional to the cube root of volume or (in our case) of weight, the regression of lactation on height confirms that of lactation on weight.

One should not, however, conclude from these results that the capacity for milk production is generally proportional to the cube root of body weight. Such reasoning would assume that the difference in lactation between our large and small cows was only the result of the difference in body size. This assumption is probably not correct. Our smaller cows were Jerseys, our larger cows Holsteins, records for both were used in this study. There was thus a difference in breed besides the difference in body size; and this suggests a possible difference in the inherent ability for lactation.

Since the records of our herd, kept under uniform conditions for 15 years, cannot be used for measuring the influence of body size on lactation, the question arises whether this relation can at all be determined empirically from data on body weight and milk production.

PREREQUISITES FOR DETERMINING INFLUENCE OF BODY SIZE ON LACTATION RATE

In any measuring of the influence of body size on lactation rate, large and small cows should be on the same food level. How can we define this food level? If a large cow gets the same amount of food, pound for pound, as a small cow, she is obviously on a lower food level than the small cow. Large and small cows may be considered on the same food level if they eat equal amounts in proportion to their respective maintenance requirements—if, for example, both take in twice or three times as much food as they need for maintenance. Instead of using the maintenance requirement as a basis, one may also use the food capacity of an animal and assume that large and small cows are on the same food level if all eat to capacity.

If small cows such as Jerseys and large cows such as Herefords are all fed to capacity and kept under otherwise equal conditions, one still could not use their lactation records for determining the influence of body size and milk production, because they differ not only in body size, but in "relative ability for lactation."

For a determination of the influence of body size and lactation, large and small cows should have the same relative ability for lactation. How can this relative ability be defined?

If two cows of equal size kept under equal conditions produce equal amounts of milk, one may conclude that they have the same relative ability for lactation. If, however, under equal conditions a large cow produces the same amount of milk as a small cow, then the large cow has a smaller relative ability for lactation than the small cow because with a larger body and, as a rule, with a greater food intake, she produces no more than the small cow.

Do large and small cows have the same relative ability for lactation when, under otherwise equal conditions, they produce equal amounts of milk energy per unit body weight, or per unit of body surface, or per unit of their maintenance requirement or their total food intake? This question must be decided before we can select large and small cows for measuring the influence of body size on lactation rate. *In short, we should know the relation of body size and lactation rate before we can measure it.*

MEASURING RELATIVE LACTATION ABILITY AS RELATIVE LACTATION CAPACITY

The theory discussed at the beginning of this paper permits us to break the vicious cycle of lactation ability and influence of body size on lactation rate because it allows an independent derivation of the second unknown. This derivation is based on the postulate that cows with equal relative lactation ability yield under equal conditions milk energy in proportion to their metabolic body size; their milk energy yield per unit of the $^3/_4$ power of body weight (kg.$^{3/4}$) is the same.

The daily milk energy per kg.$^{3/4}$, which may be classified as *relative lactation level*, permits us to compare the production rate in large and small cows independent of body size. The relative lactation level measured under standardized environmental conditions, especially of food, is an index of the ability of cows to produce milk, and the vague "ability" may thus be replaced by a clear concept, *the standard relative lactation level*, which is defined as a measurable quantity. If the standard conditions are close enough to optimal conditions for lactation and if the lactation level is measured over a long enough time—for example, a whole lactation period—then the result should be an index of the inherent capacity for milk production. The *relative lactation capacity* may thus be defined as the energy in milk yielded during a given time under standard and quasi-optimal conditions expressed per day and per unit of the $^3/_4$ power of body weight.

The relative lactation level under the conditions at our Station for first-lactation cows milked thrice daily throughout 10 months was 125.5 ± 2.8 kilocalories of milk energy per day per kg.$^{3/4}$ for the 24 Holsteins and 139.0 ± 4.8 kilocalories per day per kg.$^{3/4}$ for the 42 Jerseys mentioned in this paper. The difference between the relative lactation levels of Jerseys and Holsteins is highly significant since its random probability is below 1 per cent.

If we can assume that the conditions at our Station were not far from optimal, then the ratio of the relative lactation levels indicates also the ratio of the respective relative lactation capacities of the two groups of cows and leads us to conclude that the relative lactation capacity of the Jerseys used in this study is 11 per cent greater than that of the Holsteins.

If, in breeding dairy cows, the selection is made according to absolute yields in milk or butterfat, there is a danger that among cows differing widely in size, the larger ones will be preferred. These larger cows may, however, not be better food utilizers, because their higher production may be and as a rule will be, at the expense of a correspondingly higher food intake. It would be preferable to select cows according to their relative lactation capacity as defined in this paper. This would be a considerable step toward breeding for efficiency of food utilization, which should be the basic criterion for selecting farm animals.

CONCLUSIONS

The theory that the capacity for rate of production in animals is in general proportional to their rate of metabolism, or in turn to the $^3/_4$ power of their body weight, is the most simple expression of the relation of body size and food utilization, since it does not involve an assumption that food value depends on body size. The theory has been rather well confirmed for milk production.

Recent results on Czechoslovakian cows, if averaged according to their statistical weight based on number of observations agree better with the $^3/_4$ power rule of productive rate than with Gaines' theory that the capacity for milk production is proportional to body weight itself. An equation by which Gaines summarizes the relation of body weight and milk yield in 32 American Holstein cows also agrees better with the $^3/_4$ power rule than with Gaines' own theory, which theory would lead to a serious complication in the formulation of food utilization.

The lactation rate in terms of milk energy yield of 24 Holstein and 42 Jersey cows kept under quasi-identical conditions at the California Experiment Station was practically independent of body size within each breed and was nearly proportional to the height at withers and to the cube root of body weight if the data of all 66 cows were analyzed together, as was done by applying the method of least squares to the logarithms of heights, weight, and milk-energy yield.

The general relation between body size and lactation rate cannot be strictly derived from empirical data alone, such as records on milk yield and body weight. For the measurement of this relation it is not sufficient that large and small cows be kept on relatively equal food and management; it is also necessary that they have on the average the same relative ability for lactation. Such ability in large and small cows can be compared only if the relation of body size to lactation rate is already established.

Our general theory on energy utilization leads to the postulate that large and small cows have an equal relative ability for lactation if, under otherwise identical conditions, their lactation rate per unit of the metabolic body size, or the ¾ power of body weight, is the same. Ability for lactation may thus be measured as relative lactation level, which is defined as milk-energy yield per day per unit of the ¾ power of body weight; or as relative lactation capacity—the relative lactation level measured over an entire lactation period under standard quasi-optimal conditions.

Provided that the conditions at our Experiment Station can be regarded as sufficiently optimal, the relative lactation capacity of the 42 Jersey cows whose records are discussed in this paper is 139 kilocalories of milk-energy per day per unit of the ¾ power of body weight in kilograms. The corresponding relative lactation capacity for the 24 Holstein cows is 125 kilocal. per kg.^{¾} per day.

To use relative lactation capacity instead of absolute yields as a criterion for selection in breeding dairy cows would be a considerable forward step toward selecting of cows for efficiency of food utilization.

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A DEVICE FOR MEASURING THE BODY OF CREAM CHEESE

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During a study of the physical properties of cream cheese it became necessary to measure the consistency or body of the different samples. Several methods which had been used for measuring the consistency of butter were tried without success. Among these methods were penetration tests based on a falling ball or needle (1) and a crushing test (2). The apparatus of Valentine and Sargent (3) was also investigated. The most satisfactory method found was a modification of the method and apparatus described by Sommer and Matsen (4) for the measuring of curd strength in milk. Their apparatus was modified by substituting a specially designed plunger for the curd knife of their apparatus.

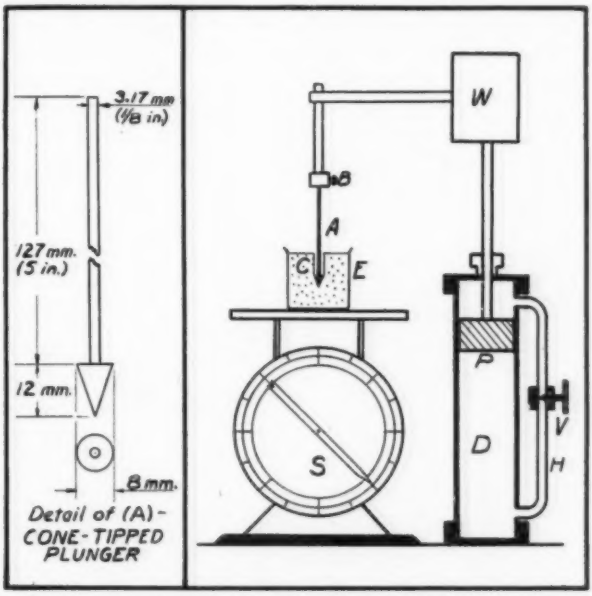

FIG. 1. Device for measuring "body" of cream cheese.—The cream cheese (*C*) packed tightly in a 100 ml. beaker (*E*) is supported on scales (*S*) which record the pressure exerted by the cone-tipped plunger (*A*). The pressure is supplied by a weight (*W*) to which the plunger is attached by means of a side arm connection (*B*). The speed of the downward movement of the weight and plunger is regulated by a needle-valve (*V*) which adjusts the rate of air flow forced by piston (*P*) through by-pass (*H*) back into the cylinder (*D*) above the piston.

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The plunger devised consists of a cone to the center of the base of which is attached the end of a drill rod one-eighth inch in diameter and five inches long. Plungers are made with cones with diameters of 4, 8, and 10 mm. Each cone is made so that the altitude is equal to one and one-half times the diameter of the base. The resistance offered by the cheese when these cones are forced into it is measured in grams in the usual manner described by Sommer and Matsen (4).

The interesting difficulties encountered in developing the dimensions of the plungers prompt us to submit these details for publication. Cream cheese is a plastic substance with varying degrees of stickiness. The dimensions of the cones were adopted after many trials because they seemed to penetrate smoothly with a minimum of compression accumulating ahead of the cone. Flatter cones tend to introduce such pressure errors as evidenced by erratic readings. The diameter of the drill rod supporting the cone was adopted because it offered the necessary strength and practically eliminated the drag occurring when rods of larger diameters touched the cheese.

Resistance of cheese to the passage of the plunger is affected not only by the size of the plunger but also by such factors as temperature of cheese, time and temperature of holding after manufacture, and care exercised to exclude air holes in packing the cheese into the container for testing. Samples to be tested are taken immediately after pressing, packed in 100 ml. beakers,

TABLE 1

Effect of cone diameter on resistance to penetration

Lot No.	Cone diameter			Lot No.	Cone diameter		
	4 mm.	8 mm.	10 mm.		4 mm.	8 mm.	10 mm.
	gms.	*gms.*	*gms.*		*gms.*	*gms.*	*gms.*
1	18	54	100	5	22	54	122
	18	57	106		21	53	130
	19	54	108		21	53	132
	19	57	.....		18	52	125
	20	55	.....		16	58	.....
2	24	66	108	6	28	73	170
	24	66	110		28	74	180
	23	72	102		29	72	175
	22	74	108		29	74	185
	21	70	108		27	80	180
3	33	105	250	7	42	105	392
	32	106	256		42	107	408
	31	102	260		42	102	408
	32	98	.....		43	119	390
	32	100	.....		40	118	.....
4	52	96	141	8	88	244	.....
	46	98	161		93	244	.....
	50	95	148		87	256	.....
	49	.....	144		90	260	.....
	.....	.....	160		.....	.....	.....

covered with aluminum foil to prevent drying and are then placed in an incubator at 72° F. for a period of 12 hours.

The relation between the measurements obtained when cones of different sizes are used on the same samples of cheese and the degree of uniformity of repeated measurements on identical cheese are shown for eight samples of cream cheese in table 1.

The data presented in table 2 show the differences in body measurements of a series of eight different lots of cheese. These lots are classified by the opinion of judges as being hard, medium hard and soft.

TABLE 2

Quadruplicate measurements of body in hard, medium and soft cream cheese. (An 8 mm. cone was used in all tests)

Hard bodied samples		Medium bodied samples			Soft bodied samples		
1	2	3	4	5	6	7	8
gms.	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*
100	110	77	56	80	36	34	32
108	110	79	57	80	34	38	33
108	107	78	58	76	40	32	33
103	110	76	57	84	40	36	36

This means of measuring the body or consistency of cream cheese affords a new method of observation for control or experimental purposes. The test is simple, sensitive, easily and quickly performed, requires little equipment, only a small amount of cheese and the cheese is not injured for sale.

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A TECHNIQUE FOR PERFUSING EXCISED BOVINE MAMMARY GLANDS[1]

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Advantages of perfusion of the surviving mammary gland in a study of milk secretion was set forth by Petersen, Shaw and Visscher (1). Since then a considerable number of experiments have been conducted in studying the activity of the perfused excised gland under varying conditions and more confidence has been established in the method as a valuable tool in the study of the phenomena involved in milk secretion. In these studies a perfusion apparatus and certain techniques have been developed which it shall be the purpose of this paper to describe.

To expedite treatment the material lends itself to the following divisions:

1. A description of the apparatus.
2. Excising and care of the excised gland.
3. Care and treatment of the blood.
4. Hook up of the gland in the apparatus.
5. Some important considerations in perfusion of the excised mammary gland.

DESCRIPTION OF THE PERFUSION APPARATUS

The perfusion apparatus now in use is reproduced according to scale in figure 1. The essential features of this apparatus are: a reservoir for the blood, a pulsating mechanism, provisions for regulating the blood pressure, provisions for warming the blood, provisions for suspending the excised gland, mechanism for oxygenating the blood and provisions for elevating the blood into the reservoir. While many variations in the structure may be made to perform the essential function of the apparatus, a description will be given of each of the parts of the apparatus now in use.

The reservoir (6 in Fig. 1) is an inverted five-gallon water bottle with three holes in the bottom. The reservoir is elevated so that the blood flows by gravity to the pulsator. A valve (4 in Fig. 1) intervenes between the reservoir and the pulsator so as to prevent backward flow of the blood upon the pressure stroke of the pump. The valves used throughout in the apparatus are illustrated in figure 2.

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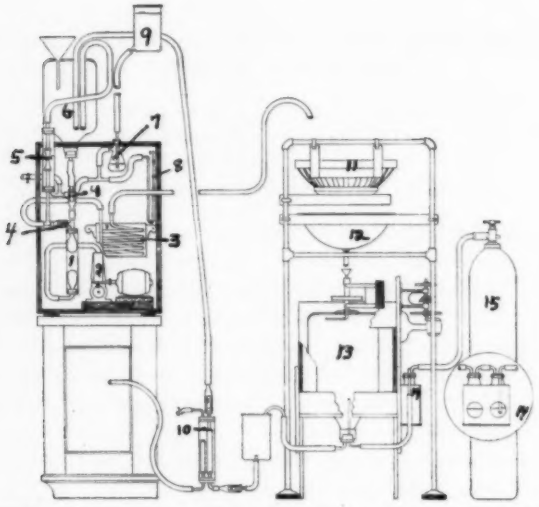

FIG. 1. Sketch of the perfusion apparatus drawn to scale. Description is given in the text.

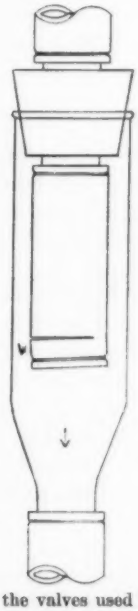

FIG. 2. Showing the details of the valves used in the perfusion apparatus. It consists of a thistle tube into which a rubber tube is inserted through a stopper. The rubber tube is plugged in the free end and cut transversely to give the proper tension.

The pulsating mechanism or "heart" (1 in Fig. 1) consists of a glass cylinder, 20 or more cm. long and 5 cm. in diameter, stoppered in both ends and filled with physiological saline. Through the lower stopper an 8-mm. glass tube is passed and on the inside is affixed a toy balloon. The outside end of the glass tube is connected to a closed air pump (2 in Fig. 1) alternating pressure and suction. A side tube equipped with an adjustable clamp is taken off from the tube connecting with the pump to permit varying amounts of air to pass in and out with the strokes of the pump and thus enable regulation of the volume output of the "heart." The pump has a capacity of 40 cc. per stroke.

The upper stopper is fitted with two glass tubes, one to permit filling or removal of the saline solution and the other a 10-mm. T tube. One branch of the T passes through the stopper and on the inside has another toy balloon affixed. Another branch of the T tube is connected with the tube coming from the reservoir while the third connects with a second T tube with a second valve (4 in Fig. 1) intervening. One arm of the second T tube connects with the resistance mechanism and the other leads to the mammary gland through the water bath.

The resistance (5 in Fig. 1) for regulating blood pressure with mercury manometer attached (8 in Fig 1) is conventional except to avoid fluctuations in pressure due to change in the temperature of the air. The desired air pressure is maintained by a water column. An aspirator (9 in Fig. 1) is filled with water and by means of rubber tubing is connected to a suction flask (7 in Fig. 1) communicating with the air chamber of the resistance. The aspirator is then raised or lowered to produce the desired pressure.

The blood is warmed immediately before going into the gland by going through a glass coil (8 mm. in diameter) immersed in a water bath (3 in Fig. 1).

The unit for perfusing the gland and oxygenating the blood is contained in a frame 72 inches high and 20 by 30 inches in the other two dimensions and made of $\frac{3}{4}$-inch iron pipe. From this frame the gland may be suspended by the median suspensory ligament and the skin or placed in a basket (11 in Fig. 1) with openings in the bottom for the teats to protrude. The latter method is preferred as provisions are made so the basket can be placed in either of two levels. At the lower level it is more convenient to make the necessary attachments in the vascular system and at the higher level the milking procedure is facilitated. (In Fig. 1 the basket is at the higher level.)

Below the suspended gland is a vessel (12 in Fig. 1) for collecting the venous blood as it comes from the perfused gland. This vessel is of metal heavily coated with paraffin. In the bottom is an opening in which is fitted a 12-mm. glass tube equipped with a rubber tube and adjustable clamp on the free end.

Immediately below the vessel for collecting the blood is the apparatus for

oxygenating the blood. This consists of a 10-gallon glass bottle (10 in Fig. 1) with provisions for distributing the blood in thin layers on the inner surface and inlets for oxygen. The glass bottle is inverted and has a hole drilled in the bottom through which is fitted an 8-mm. tube bent at right angles and extending within one inch from the side walls of the bottle. The protruding end of the glass tube passes up through a metal tube fixed in a pulley and fitted in bearings so the whole can be rotated to distribute the blood on the inner walls of the glass bottle. To the upper end of the glass tube is fixed a glass funnel or thistle tube into which the blood passes as it comes from the collecting vessel.

The lower opening of the inverted glass bottle is stoppered with a rubber through which passes two glass tubes, one for the introduction of the oxygen and the other serves as an outlet for the oxygenated blood. The oxygen is saturated with moisture by passing through two bottles partly filled with water. Details of this are given in the inset (14 in Fig. 1).

The oxygenated blood is passed through a cotton filter after it comes out of the glass bottle and flows by gravity to the pump (10 in Fig. 1) which elevates it to the reservoir previously described. The pump is constructed of a two-inch glass tube similar to the "heart" in which the essential difference is that instead of the toy balloon incoming glass tubes are connected with Gooch rubber tubing, permitting the blood to flow through the entire length of the pump. Pulsation action is furnished by the same type of pump as is used for the "heart." Valves on each end of the pump permit flow in but one direction.

EXCISING AND CARE OF THE EXCISED GLAND

In removing the gland from the slaughtered cow extreme care must be exercised in avoiding any injury to the glandular tissue. It is preferable, although not essential, to leave the entire gland covered with skin. In making the incision anterior to the gland care should be taken to leave two or more inches of the subcutaneous abdominal veins. It is also desirable to have long external pudic veins and arteries. This is secured by drawing them out of the inguinal canal before severing. The gland is more easily excised when the animal is on its back.

After the gland is excised it is thoroughly washed in warm physiological saline and the external pudic arteries freed of any blood they may contain. Any blood clots in the arteries will cause occlusion of parts of the gland during perfusion.

CARE AND TREATMENT OF THE BLOOD

For the experiments to date the blood has been secured either by carotid bleeding of animals slaughtered at University Farm or by the usual bleeding method in packing-house slaughter. By the former method arterial blood

and by the latter method mixed blood is secured. The hemoglobin of the blood secured from the packing house material is usually much reduced because of dyspnea caused by stunning and therefore requires oxygenation before it can be used for perfusion.

To date four methods have been employed to prevent clotting: defibrination, addition of chlorazol fast pink, sodium citrate and heparin.

In defibrinating, a wooden fork is used to stir the blood immediately after it is drawn. Care is taken not to stir too vigorously or injury to the cells results, yet stirring must be vigorous enough to secure a relatively clear and firm fibrin or too much loss of blood will result from inclusion in the clot.

From experience it has been found that one gram of chlorazol fast pink per liter of blood is essential to prevent clotting for 24 hours and give the necessary safety factor. The chlorazol fast pink is dissolved in physiological saline at the rate of 12 grams to 100 ml. saline solution. This is placed in the container before the blood is drawn and stirred to insure prompt mixing.

For certain experiments sodium citrate may be used as an anticoagulant. Approximately five grams of sodium citrate are needed per liter of blood. A 60 per cent solution of the salt has been found to be satisfactory when added in a manner similar to that described for chlorazol fast pink.

Heparin is the ideal anticoagulant except for expense. It should be added according to directions given by the manufacturers, or if made in the laboratory it should be tested for its anticoagulating powers and then added 50 per cent in excess over the amount required to prevent blood from coagulating in 24 hours.

After the blood has been treated to prevent coagulation an important step is thorough and careful filtering to remove any clots or any foreign particles with which it may be contaminated. Failure to thoroughly filter out all material will ruin a perfusion experiment. For adequate and most rapid filtering a series of three filters are used arranged one above the other. For the top layer three or four layers of cheese cloth are used to catch the coarser particles. The second filter consists of double the number of layers and the third filter consists of cotton inserted between two layers of cheese cloth. All filters are wetted with physiological saline before being used.

The next step, except for blood drawn from the arteries, which is ready for use, is to oxygenate the blood. This is done by passing it through the oxygenator previously described.

HOOK-UP OF THE EXCISED GLAND

After the gland has been thoroughly cleaned it is either suspended in the apparatus from the skin and median suspensory ligament or placed in the basket previously described. The external pudic artery is carefully freed from the vein, nerve and lymph vessels with which it is associated and a glass cannula of the proper size connected to the pulsating system and filled with

warm blood is inserted. In making this connection care must be taken that the artery and blood system is free from air or air embolism in the gland will result.

As small clots in the veins and bits of tissue from the cut surface will contaminate the first blood through the gland, the first several liters should be collected and filtered through the original series of filters before being again put into the system.

As there are many venous escapes from the udder of a cow, blood will flow out from many places. It is usually advisable to have the flow from but one outgoing vein and as all veins communicate with the venous circle all but the desired outlet may be ligated.

From the experience gained in more than 70 perfusions a number of fundamental essential techniques have been observed, the more important of which will be set forth.

Avoid metal contact with the blood. It was observed that when the blood came in contact with certain metals it developed vasoconstrictor properties. In the perfusion apparatus all parts with which the blood comes in contact are of glass, rubber, cotton for filters or paraffin used in coating metal containers. The vessel into which the blood is drawn from the animal is thoroughly coated with paraffin.

Time between slaughter and beginning of the perfusion. The length of time that may elapse between slaughter and the beginning of the perfusion and have the gland function normally is not as yet too well established. In experiments to date this time has varied from seven minutes to two hours. When two hours elapse much damage is done to the gland tissue. No difference has been observed when the intervals were from seven to forty-five minutes.

Avoid rough handling of the gland. While no studies have been conducted as to the effect of stimulating the nerve trunks upon the rate of blood flow through the gland it has been noted that whenever the gland was handled carelessly in cannulating arteries or veins, or ligating veins, a marked lowering in blood flow resulted. It is postulated that this effect is the result of stimulation of nerves, causing vasoconstriction.

Filtering blood. For a successful perfusion it is imperative that the blood be filtered through a cotton filter before entering the blood reservoir. Failure to do this will result in a progressive slowing up of the blood flow through the gland because of occlusion to the smaller vessels by particles in the blood.

Emptying the gland of milk. At the beginning of a perfusion it is essential to have the gland emptied of any milk accumulated therein in order that the milk secreted during a perfusion may be accounted for. Immediately after the blood starts through the gland one ml. of obstetric pituitrin is injected into the inflowing blood. As the teats are previously cannulated this

causes an expression of all of the milk present in the gland. As the obstetric pituitrin contains considerable of the pitressor principle the first few hundred ml. of venous blood following the injection are discarded or some vasoconstriction may be expected.

At the completion of the perfusion obstetric pituitrin is again administered and the milk secreted during the perfusion is expressed. Cannulae are kept in the teats during the perfusion to permit drainage of all milk remaining in the ducts and thus to avoid such milk from contaminating that produced during the experiment.

Blood pressure. Shaw and Petersen (2) have shown that the blood may either become concentrated or diluted in passing through the mammary gland. Changes in blood pressure will produce such changes and invalidate any observations upon arteriovenous difference for blood constituents. A blood pressure of 120 mm. Hg has been found satisfactory.

Establishing venous pressure. Venous pressure of any level may be established by cannulating a vein and elevating the outlet to the desired height. A 10-mm. glass U tube with a hole blown in the curve may be attached to the cannula by rubber tubing to facilitate carrying off the venous blood.

Taking blood samples. Arterial blood samples are taken from the tube leading into the external pudic artery by means of a hypodermic needle. Venous samples are taken from the overflow U tube. Venous samples for CO_2 and O_2 analyses may be taken by insertion of a long hypodermic needle through the hole blown in the U tube.

Adding material to the blood. Depending upon the type of study to be made, different materials are to be added to the blood. To maintain the glucose level of the blood 10 mg. of glucose must be added to each 100 ml. of blood passing through the gland. To avoid dilution of the blood, glucose is added in a 50 per cent solution. Calcium, fats, amino acids and other constituents may be added.

Length of a perfusion experiment. There is no fixed time to which a perfusion should be adjusted. As a matter of routine six hours is the time that has been adopted and which is a long enough time to study the various factors under consideration.

SUMMARY

An apparatus for the perfusion of the excised bovine mammary gland is described.

Directions are given for excising and care of the excised mammary gland, taking of the blood, use of anticoagulants and care of the blood.

The more important considerations in conducting a perfusion experiment are also treated.

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SOME CHEMICAL CHANGES OCCURRING IN MOLASSES GRASS SILAGE*

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Many crops, such as the grasses and legumes, when made into silage, require the addition of a preservative at the time of ensiling to increase the acidity of the ensiled mass to a pH of about 4.0, a point at which undesirable bacterial activities are inhibited. The commonly used preservatives fall essentially into three general groups. One method involves the addition of inorganic acids to the fodder, causing an immediate increase in the acidity. A second method is the addition of a source of fermentable carbohydrate such as molasses which, through acid fermentation, may cause a more gradual increase in the acidity of the mass. The third method is a combination of the other two, *i.e.*, the addition of both inorganic acids and fermentable carbohydrates; the amount of inorganic acid added is insufficient to completely preserve the silage, the complete preservation being dependent on an acid fermentation of the carbohydrates.

The addition of a source of fermentable carbohydrate such as molasses does not give an immediate increase in the acidity of the fodder. It seemed desirable, therefore, to attempt to follow the progress of the fermentation. The determination of the rate of sugar disappearance as correlated to the increase in acidity should give an indication as to the time required to reach the optimum acidity for preservation and also may give a basis for the calculation of the amount of fermentable carbohydrate needed to reach this optimum acidity and the nutrient loss involved in the fermentation.

PROCEDURE

During the summer of 1939 a study was conducted at the Dairy Research Farm of the New Jersey Agricultural Experiment Station at Sussex, New Jersey. On July 17, thirty-three tons of a mixture of field peas and oats were ensiled with the addition of approximately one hundred pounds of molasses per ton. Samples were taken periodically from the silo through holes drilled in the silo wall. Because of the capacity of the concrete pit of the silo, it was necessary to take the samples about five feet below the top level of the silage. The borings were spaced about two feet apart around the silo and in such a way that by allowing for the settling of the silage, the samples were taken from the same layer in the silo. The holes were plugged with wood immediately after withdrawal of the sample.

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Each sample which consisted of about ten kilograms was mixed thoroughly and ground by means of a power meat chopper. After thorough mixing of the ground material, aliquots were taken for the determination of pH, volatile acids, total sugars, reducing sugars, total nitrogen, ammonia nitrogen, water-soluble nitrogen, and during the later stages of the fermentation, lactic acid.

The pH was determined electrometrically by means of the quinhydrone electrode. The acetic, butyric, and lactic acids were determined by the method of Flieg (1). As sucrose cannot be removed from the water extract and as it affects the lactic acid determination, no lactic acid values were determined for the first two weeks. The values which are recorded for lactic acid are probably slightly too high, as a result of the presence of some sucrose, which was assumed to be the difference between the values for total and reducing sugars. Total nitrogen was determined on the fresh sample; and water-soluble nitrogen, on a water extract of the silage. The ammonia nitrogen was determined by the distillation of a water extract of the silage made alkaline with magnesium oxide, which, being relatively insoluble, releases the ammonia but does not permit the distillation of other volatile nitrogen bases such as would distillation from a sodium hydroxide solution. Sugars were determined by the method of the A. O. A. C. for sugars in feeds.

RESULTS

The results of the analyses are given in table 1. The periodic changes in pH, acetic acid, and total sugars are shown graphically in figure 1.

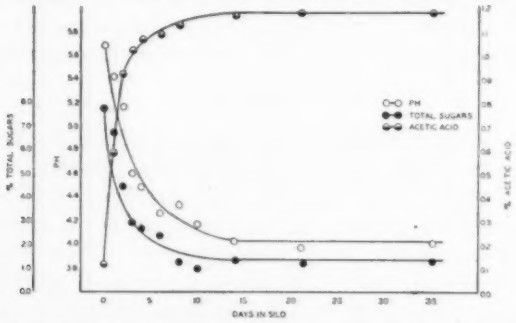

FIG. 1. Periodic changes of pH, total sugars, and acetic acid in molasses grass silage.

During the first 5 days there was a rapid decrease in pH and total sugar content followed by a slower decrease of the same for about 10 days after which there was no significant change. The increase in acetic acid content was at about the same rate as the decrease in pH and total sugars.

The periodic determinations of total nitrogen, water-soluble nitrogen,

TABLE 1

*Chemical changes occurring in oats and pea molasses silage**

Date	Days in silo	pH	% Acetic acid	% Butyric acid	% Lactic acid	% Total sugars (as glucose)	% Reducing sugars (as glucose)	NH_3 – N % total N	Water sol. N % total N	Total N
July 17	0	5.69	0.12	None	.....	7.74	2.40	None	20.9	0.54
July 18	1	5.42	0.59	– 0.11	.....	6.76	3.58	0.02	67.1	0.54
July 19	2	5.16	0.92	– 0.10	.....	4.48	3.26	0.03	72.8	0.56
July 20	3	4.60	1.02	– 0.11	.....	2.93	2.09	0.02	55.9	0.76
July 21	4	4.49	1.07	– 0.09	.....	2.68	2.28	0.02	.....	0.71
July 23	6	4.27	1.09	– 0.03	.....	2.40	2.09	.....	.....	.....
July 25	8	4.34	1.13	– 0.01	.....	1.28	1.08	.....	.....	.....
July 27	10	4.18	.....	.....	.....	1.00	0.89	0.03	42.9	0.78
July 31	14	4.04	1.17	None	2.00	1.37	0.67	0.03	.....	0.74
August 7	21	3.98	1.18	None	2.25	1.23	0.85	0.02	49.3	0.68
August 21	35	4.03	1.18	None	2.27	1.33	0.99	.....	.....	.....

* All analyses were made on the fresh silage.

and in the latter stages of the fermentation the sugar and acidity determinations demonstrate a rather great source of error, which might be called a sampling error. During the early stage of the fermentation the rate was undoubtedly rapid enough to mask this error with respect to the increase in the acidity and the disappearance of the sugars. The total sugar content from the eighth day on shows a variation which can only be attributed to the sampling technique. As the layer sampled was not a homogeneous mass it seems reasonable to conclude that although the data presented show the trend of the carbohydrate fermentation, the values given are not absolute in showing the exact changes in composition during the fermentation in silage. Before any definite conclusions are drawn more data are necessary.

The determinations of the water-soluble nitrogen are meaningless with respect to the determination of the degree of protein breakdown during the fermentation. It is interesting to note, however, that the percentage of ammonia nitrogen remains essentially constant during the entire period of the experiment. This would seem to indicate that there is no deamination of the proteins or amino acids during the course of the fermentation. This possibly may be explained on the basis that the carbohydrate available to the bacteria is an adequate source of energy. When sufficient carbohydrate is available to the bacteria as an energy source, the utilization of protein for energy is suppressed, only sufficient protein being utilized to supply the nitrogen requirements of the bacteria. This possibly could be determined more satisfactorily by conducting a similar study in a silo in which there is an inadequate carbohydrate supply to give an increase in acidity to pH 4.0, at which point proteolytic bacteria and their enzymes are inactivated.

The negative values listed for butyric acid possibly may be attributed to the formation of formic acid as a carbon dioxide precursor, rather than to the inaccuracy of the method of the determinations. It may be assumed that during the period of the rapid fermentation formic acid is formed faster than it is broken down to carbon dioxide and hydrogen, the hydrogen undoubtedly taking part in another of the simultaneous reactions. The volatile acids were determined by distillation. In such a method, if formic acid is present it will have the effect of lowering the apparent butyric acid content and increasing the acetic acid content. At no time during the fermentation could the odor of butyric acid be noted. It would seem incorrect, therefore, to assume that the lowest negative value is the zero value and that all the other values indicate the presence of butyric acid. Further study demonstrating the presence of formic acid during the early stages of the fermentation will be necessary to verify this contention.

It may safely be said that under the conditions of this experiment the most rapid fermentation occurred during the first week. It is to be emphasized, however, that although the general trend of the fermentation in this experiment may also apply to other cases under different conditions with

regard to the crop ensiled and the location in the silo, where undoubtedly the effects of temperature and pressure vary, these results are not necessarily a picture of the absolute rate or trend of the fermentation in all cases of crops ensiled with the use of fermentable carbohydrates as a preservative. The clarification of this problem will require further study.

SUMMARY

The periodic determinations of pH, total sugars, reducing sugars, volatile acids, total nitrogen, water-soluble nitrogen, and ammonia nitrogen in a silo containing a mixture of oats and field peas preserved with approximately one hundred pounds of molasses per ton of green material showed that the major portion of the fermentation process occurred during the first week.

As certain of the data show the silage to be a heterogeneous mass, the inherent errors involved in the sampling technique must be taken into consideration in the interpretation of these and other results in silage research.

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THE RELATIONSHIP OF ASCORBIC ACID TO REPRODUCTION IN THE COW[1]

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Since it has been found that ascorbic acid injections definitely improved a large percentage of sterile and partially sterile bulls (3), certain data have been accumulated upon the relationship of ascorbic acid to reproduction in the cow. The results of these experiments and observations are herewith reported.

EXPERIMENTAL

These experiments were intiated for the purposes of determining, (a) the ascorbic acid content of the blood of dairy cows, (b) the influence of the estrus cycle upon ascorbic acid levels of the blood, and (c) the effect of ascorbic acid therapy upon the "hard to settle" cow.

Analyses for blood plasma ascorbic acid were made by the method of Mindlin and Butler (2). Precautions were taken to avoid direct exposure to sunlight or strong light of any kind and to keep samples properly iced if some time elapsed between the drawing of the sample and the analyses. We have found that immediately placing the blood sample on ice in a thermos jug preserves the ascorbic acid satisfactorily for periods up to 12 hours.

The first experiment consisted of ascorbic acid analyses upon the blood plasma of five cows which had been bred from three to seven times without conceiving, and a comparison of these values with an equal number of observations from good breeding cows selected from the University herd. In this experiment it became evident that there was considerable variation in the blood ascorbic acid level of individual cows. It also appeared that cows in estrum showed an elevated blood ascorbic acid, that their blood was higher on the average in ascorbic acid than bulls of the same breed, and that there was a tendency toward breed difference.

In order to test these preliminary observations further detailed observations were made upon as many suitable cows as became available. An average value for ascorbic acid content of cow blood was found to be approximately 0.39 mgs. per cent, with the range of values lying between 0.19 and

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0.65 mgs. per cent. Ascorbic acid analyses on certain breeds are summarized in table 1. These data represent analyses made upon the University

TABLE 1

Plasma ascorbic acid of dairy cows by breeds examined from February to April, 1940

Breed	No. of cows	Plasma ascorbic acid (mgs. %)	
		Range	Average
Holstein	65	.192–.594	.353
Guernsey	68	.186–.622	.408
Jersey	15	.234–.626	.453
Brown Swiss	6	.414–.656	.534
	154 cases		.394

herd and several farm herds variously located throughout the State. It is evident that there was a distinct difference in blood ascorbic acid values among breeds. In general the Guernsey breed requires higher concentrations to maintain normal breeding performance than do the Holsteins. It appears that the ascorbic acid values of cow plasma was somewhat higher than the bull. The average blood plasma ascorbic acid value for 22 potent bulls with good breeding records was found to be 0.27 mgs. per cent. This represented 7 Holsteins, 8 Guernseys, 5 Jerseys and 2 Brown Swiss bulls.

TABLE 2

The influence of estrum upon the plasma ascorbic acid of the cow

Cow	Date	Time	Plasma C	Remarks
640	2/23/40	8: 00 A.M.	.234	Not in estrum
	3/15/40	10: 30 A.M.	.636	In estrum in the morning
Jersey	" " "	3: 00 P.M.	.546	Bred at 4: 30 P.M.
	" " "	8: 00 P.M.	.492	Going out
	3/16/40	4: 00 A.M.	.150	Estrum completely over
	" " "	8: 00 A.M.	.150	" " "
39	1/10/40	9: 00 A.M.	.270	Diestrum
	1/11/40	9: 00 A.M.	.330	"
Holstein	1/12/40	9: 00 A.M.	.247	"
Heifer	1/22/40	9: 00 A.M.	.296	"
	1/24/40	5: 00 P.M.	.378	In estrum
	1/25/40	10: 00 A.M.	.510	" " (late)
	1/30/40	4: 30 P.M.	.340	Diestrum
404	2/23/40	10: 00 A.M.	.428	Diestrum
Guernsey	4/1/40	10: 00 A.M.	.680	In estrum
91	2/23/40	10: 00 A.M.	.210	Diestrum
Holstein	3/26/40	10: 00 A.M.	.333	Estrum
40	1/8/40	9: 30 A.M.	.620	Estrum and bred at 10 A.M.
	1/9/40	9: 00 A.M.	.384	24 hrs. after estrum
Holstein	1/10/40	9: 00 A.M.	.540	Diestrum
Heifer	1/11/40	9: 00 A.M.	.364	"
	1/12/40	9: 00 A.M.	.350	"
	1/17/40	9: 00 A.M.	.400	"
	1/19/40	9: 00 A.M.	.360	"
	1/22/40	9: 00 A.M.	.420	"

Studies to ascertain the effect of estrum upon the vitamin C content of blood plasma were made. In most cases cows with a regular and consistent breeding history showed results similar to those indicated in table 2. As compared to diestrum there was a rise in ascorbic acid which reached a peak in mid to late estrum. It was difficult to always obtain samples from the cows at the proper time and the rise in ascorbic acid concentration has not, therefore, been a constant phenomenon. Enough clear-cut cases have been obtained to lead us to believe that the ascorbic acid concentration of blood plasma increases for a short period during the height of estrum and any failure to detect the change is attendant upon the difficulties involved in accurately ascertaining estrum and its various stages. The concentration at times and with certain cows has increased as much as 75 per cent or more. It is interesting in this connection that certain "hard to settle" cows did not show any increase in plasma ascorbic acid at the time of estrum (table 3).

TABLE 3

Summary data of plasma ascorbic acid values from cows classified as to breeding record

Cow	Ascorbic acid (mgs. %)		Breeding record
	Diestrum	Estrum	
667	.376	.460	Good
40	.402	.620	"
39	.296	.378	"
146	.378	.454	"
412	.420	.466	"
91	.210	.333	"
661	.456	.564	"
660	.596	.572	Poor
640	.234	.636	"
662	.322	.362	"
404	.428	.680	"
1450	.184	.284	"
1479	.262	.330	"
17	.540	.514	"
Ave.	.364	.475	

On the basis of the foregoing results "hard to settle" cows were sought and treated by ascorbic acid injections. Particularly noticeable was the case of cow No. 17, a cow fed entirely on a dry ration. This cow calved normally and 9 weeks later began to have normal heat periods at regular intervals of 21 days. Ten such periods of estrum followed. During this interval she was bred during 8 of the 10 periods. When it became evident that she was hard to settle, various maneuvers were tried in the hope of obtaining a conception. She was bred early and late in the period and on one occasion every four hours throughout estrum and all without success. Ascorbic acid analyses were then made on her blood plasma. Her breeding record is shown in table 4. The blood C values were abnormal in that no peak concentration was obtained during estrum. On February 12, 1940, she was in heat and at that time she was given 0.5 gram of ascorbic acid

TABLE 4

Data showing the effect of ascorbic acid therapy upon hard to settle cows

Cow	Before treatment (No. times bred)	Type of case	After treatment		Method of treating
			Services	Result	
427	4	Regular estrum—failed to settle	1	Pregnancy	Injections 8 wks. before breeding—wkly., subcutaneous
455	7	Skips 1 or 2 periods after service	1	"	6 injections over 5 wks. beginning with breeding
437	6	Skips 1 or 2 periods after service	1	"	6 injections over 30 days prior to breeding
17	8	Regular estrum—failed to settle	1	"	Intravenous and subcutaneous injection at time of heat
16	9	Regular estrum—failed to settle	1	"	4 doses over 10 days after breeding
29	4	Skips 1 or 2 periods after service	1	"	4 doses over 10 days after breeding
33	5	Skips 1 or 2 periods after service	1	"	4 doses over 10 days after breeding
1W	6	Regular estrum—failed to settle	1	"	3 doses over 12 days before breeding
9W	5	Regular estrum—failed to settle	1	"	3 doses over 12 days before breeding
4W	4	Regular estrum—failed to settle	1	"	3 doses over 12 days before breeding
1450	Many	Uterus—no tone	Many	No help	Subcutaneous and intravenous
1479	"	" " "	"	" "	" " "
B	4		3	?	Subcutaneous
65	Many	Cystic ovary	Many	No help	"
404	"	" "	"	" "	"
662	7	" "	Several	" "	"
D	Many	Regular estrum—failed to settle			" " "
					Dosage too low

intravenously and 2 grams subcutaneously. She was immediately inseminated and conceived from this service.

This treatment was made on the premise that the high concentration of ascorbic acid normally found in the blood during estrum functioned in the development of the corpus luteum since this body has been shown to be very rich in ascorbic acid and develops very rapidly following ovulation. This premise was not without support from other experiments. Many of the cows in the University herd presented cases which after breeding skipped the next period or two and came back in heat on the 42nd or 63rd day. Slaughter of a heifer on the 24th day after breeding showed a cystic corpus luteum which was judged to be insufficient for the long continuation of pregnancy. These cases suggested that corpus luteum formation might be at fault. Administration of ascorbic acid was sufficient to bring about diagnosable pregnancy following breeding in the majority of these cases. The data obtained from all cases treated are summarized in table 4. It is obvious that cystic ovary cases were not benefited by the treatment while the other cases described above were.

Case No. 1479 whose uterus lacked tone and seemed quite small was treated later with α-tocopherol.[2] This was administered at the rate of approximately 1.0 mg. per kg. of body weight for a period of 4 weeks. In all, 19.25 grams, dissolved in ethyl laurate, were injected subcutaneously during the course of the treatment. She was bred during this time and several times subsequently without impregnation. Following service, this heifer frequently skipped from 1 to 3 heat periods. It was quite evident that pure α-tocopherol did not improve the breeding history of this particular case.

DISCUSSION

From these observations and our experience with "hard to settle" cows it appears that certain types of cases are amenable to ascorbic acid therapy. Success has been attained when the reproductive tract of the subject was normal. Anatomical abnormalities have not been corrected. The cases which seem to be amenable to this type of therapy apparently fall into the following classes: (a) cows with regular heat periods and whose reproductive tract upon examination appears normal, and (b) cows that apparently settle on service and skip the next heat period or two before coming into heat again. Because of the high concentration of ascorbic acid in the corpus luteum, its rapid development following ovulation and its function in pregnancy it seemed that this type of case was one which involved an inadequacy of the corpus luteum. These well-known facts together with the manner in which ascorbic acid can be administered and obtain pregnancy has led us to believe that the therapy has a stimulating action upon the corpus luteum. From our data it appears that ascorbic acid can be given before breeding

[2] We are indebted to Merck and Company for the crystalline ascorbic acid and α-tocopherol used in these experiments.

and thereby saturate the organism and obtain results; it can also be given with positive results at the time of breeding if the dose is large enough or it may be given beginning with breeding and subsequent administration for several weeks. These responses suggest that ascorbic acid therapy effects either directly or indirectly the function of those processes involved in implantation and the maintenance of early pregnancy. Frequently it is possible to detect poor breeding cows because of their abnormally low plasma ascorbic acid.

We are aware of the experiments of Saffrey and Finerty (4). They came to the conclusion that abortion and resorption in the guinea pig was not due to an effect of vitamin C on the corpus luteum. More extensive experimentation is needed to clarify this point, but if their results are substantiated then a new physiologic role for ascorbic acid must be postulated.

The therapeutic effect of ascorbic acid upon the cow has been substantiated by Dr. Allan K. Brown (1). He found that ascorbic acid therapy greatly aided 12 cows which had been bred on the average 10 times each. They were given ascorbic acid injections with the result that 7 or 8 settled with the first service following treatment.

SUMMARY AND CONCLUSIONS

The results of these experiments have shown: (a) that the ascorbic acid content of plasma averaged 0.39 mg. per cent for cows of all breeds studied; (b) that there is a difference between breeds with the Holstein lower than the Guernsey; (c) that there is a higher concentration of ascorbic acid in the plasma of the cow in mid to late estrum than there is in diestrum; (d) that generally speaking there is no difference between good and poor breeders in their peak concentrations of ascorbic acid during estrum; (e) that the subcutaneous ascorbic acid therapy of "hard to settle" cows results in a positive response in 60 per cent of the cases treated; (f) that ascorbic acid therapy does not correct cases of cystic ovary or other anatomical abnormalities; and, (g) that α-tocopherol proved ineffective in restoring tone to a toneless uterus.

From these researches the conclusion is evident that ascorbic acid is intimately associated with the early phases of the reproductive processes and it can be successfully used as a therapeutic measure in treating certain types of sterility in the cow.

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A SUPPLEMENTAL NOTE ON THE CORRELATION BETWEEN FAT PERCENTAGE AND YIELD OF MILK, MILK FAT, MILK ENERGY

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In a previous article (1) it was shown, by use of regression equations,[1] that if $r_{tFCM} = 0$, $-r_{tM} = \frac{\bar{f}}{8/3 + \bar{f}} \frac{V_t}{V_M}$. The present note confirms that result, by more direct and, particularly, more inclusive methods. In fact, if $\bar{f}$, V_t, V_M and r_{tM} are known the present formulas (table 1), without any additional data, permit the computation of r_{tF}, r_{tFCM}, r_{MF}, r_{MFCM} and r_{FFCM}. After confirming the above result, first mentioned, in general terms, the purpose is to apply the formulas to a set of observed values of f and M and to discuss some of the possible biological interpretations of the statistics thus obtained.

PREVIOUS RESULT CONFIRMED

From the second equation of table 1 it is evident that if $r_{tFCM} = 0$, $\bar{f}V_t + r_{tM}(8/3 + \bar{f})V_M = 0$ and $-r_{tM} = \frac{\bar{f}}{8/3 + \bar{f}} \frac{V_t}{V_M}$. This result is the same as the one previously found (1, equation 5).[2] It remains to explain the derivation of the formulas.

The derivation of the formulas in table 1 may be explained by using the one for r_{tF} as an illustration. A general formula for the coefficient of correlation is, $r_{xy} = \frac{\overline{xy} - \bar{x}\bar{y}}{\sigma_x \sigma_y}$, where x and y symbolize the paired variables correlated.

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[1] Symbols are used as follows:

f = per cent fat in the milk yielded
M = milk yield, pounds
F = milk fat yield, pounds = .01fM
FCM = milk-energy yield, pounds 4 per cent milk = .4M + 15F = .15(8/3 + f)M
n = number of records
An overscored item = mean of the item overscored
σ_f = standard deviation of f
$V_t = \sigma_t / \bar{f}$
r = coefficient of correlation

[2] The procedure in deriving the previous result involves two approximations. Agreement of the present result with the previous one shows that the two approximations effectively neutralize each other. It was previously (1, footnote 4) noted that $\bar{f} = 4$ and $V_t/V_M = 2$ give the result, $r_{tM} = -1.2$, which was mistaken to indicate the approximations were too faulty in such a case. It really shows that r_{tFCM} cannot equal zero in such a case. In fact, if $\bar{f} = 4$ and $V_t/V_M > 5/3$ it follows from the second equation in table 1 that $r_{tFCM} > 0$.

Following this form, $r_{tF} = r_{t(fM)} = \dfrac{\overline{ffM} - \overline{ff}\,\overline{M}}{\sigma_t \sigma_{fM}}$. By use of the regular formulas for means and standard deviations for the products of 2 or 3 variables, *e.g.*, as given by Dunn (2, page 334), and bearing in mind that $\overline{f}V_f$ may be substituted for σ_f, and $\overline{M}V_M$, for σ_M, we have:

$$\overline{fM} = \overline{f}\,\overline{M} + r_{fM}\overline{fM}V_fV_M$$

$$\overline{ffM} = \overline{ff}\,\overline{M} + \overline{ffM}V_fV_f + 2r_{fM}\overline{ffM}V_fV_M$$

$$\overline{ffM} = \overline{ff}\,\overline{M} + r_{fM}\overline{ffM}V_fV_M$$

$$\overline{ffM} - \overline{ff}\,\overline{M} = \overline{ffM}V_fV_f + r_{fM}\overline{ffM}V_fV_M$$

$$\sigma_t\sigma_{fM} = \overline{f}V_f\overline{fM}[V_f^2 + V_M^2 + 2r_{fM}V_fV_M]^{.5}$$

The last equation above gives the denominator, and the second last gives the numerator in the formula for r_{tF}. Cancelling $\overline{ffM}V_f$ in the denominator and both terms of the numerator gives $r_{tF} = \dfrac{V_f + r_{fM}V_M}{[V_f^2 + V_M^2 + 2r_{fM}V_fV_M]^{.5}}$. Inasmuch as this same denominator term reappears in two of the other formulas, it is for convenience designated as $A^{.5}$ in the table. The other formulas are derived in a similar (although more complicated) way.

TABLE 1

Formulas for computation of r_{tF}, r_{tFCM}, r_{MF}, r_{MFCM} and r_{FFCM} where $\overline{f}$, V_f, V_M and r_{fM} are first computed from the observed values of f and M

$$r_{tF} = \frac{V_f + r_{fM}V_M}{A^{.5}}$$

$$r_{tFCM} = \frac{\overline{f}V_f + r_{fM}(8/3 + \overline{f})V_M}{B^{.5}}$$

$$r_{MF} = \frac{V_M + r_{fM}V_f}{A^{.5}}$$

$$r_{MFCM} = \frac{(8/3 + \overline{f})V_M + r_{fM}\overline{f}V_f}{B^{.5}}$$

$$r_{FFCM} = \frac{\overline{f}V_f(V_f + r_{fM}V_M) + (8/3 + \overline{f})V_M(V_M + r_{fM}V_f)}{A^{.5}B^{.5}}$$

$$A = V_f^2 + V_M^2 + 2r_{fM}V_fV_M, \text{ approximately}^a$$

$$B = [\overline{f}V_f]^2 + [(8/3 + \overline{f})V_M]^2 + 2r_{fM}\overline{f}V_f(8/3 + \overline{f})V_M, \text{ approximately}$$

[a] That is, neglecting the term, $+V_f^2V_M^2(1 + r_{fM})$, which is ordinarily far too small to be of any consequence in the final result.

The formulas in table 1 presuppose that the f and M values conform reasonably well to normal frequency distributions. This condition is ordinarily satisfied where f and M are taken within a breed of dairy cows and n is large. Where n is small or the distributions too irregular the r's computed by use of the formulas may not agree with the r's computed directly from the observations.

USE OF THE FORMULAS IN COMPUTATION

In a given population say we have observed values for each of the 4 variables, f, M, F, and FCM for individual cows and wish to compute the coefficient of correlation between these variables in all possible pairs. We may proceed by computing $\bar{f}$, V_f, V_M and r_{fM} in the regular way. Then by substituting the numerical values of these statistics in the literal formulas in table 1 all the other r's can be readily obtained. Where n is large this procedure effects a saving in time and labor.

TABLE 2

A comparison of the values of r_{fF} and r_{fFCM} as computed directly from the observations and indirectly by use of the formulas in table 1. (Milking Shorthorn Record of Merit Cows)

Group[a]	Mean FCM	n	Computed directly from the observations						Indirectly[b]	
			$\bar{f}$	V_f	V_M	r_{fM}	r_{fF}	r_{fFCM}	r_{fFCM}	r_{fF}
1	8292	2585	3.99	.082	.250	−.224	+.107	−.027	−.028	+.106
2	11351	345	3.88	.078	.240	−.152	+.174	+.042	+.041	+.172
3	7987	2130	3.97	.084	.219	−.219	+.169	+.012	+.011	+.166
4	9579	285	3.93	.073	.218	−.161	+.189	+.048	+.039	+.174
5	7424	504	3.97	.082	.233	−.196	+.159	+.016	+.015	+.157
6	7428	462	4.05	.092	.227	−.138	+.259	+.107	+.107	+.260
All	8282	6311	3.97	.084	.260	−.217	+.106	−.026	−.024	+.108

[a] Group 1: 2 milkings daily; in milk 12 months; no calving requirement.
Group 2: 3 milkings daily; in milk 12 months; no calving requirement.
Group 3: 2 milkings daily; in milk < 12 months; no calving requirement.
Group 4: 3 milkings daily; in milk < 12 months; no calving requirement.
Group 5: 2 milkings daily; in milk 10 months; calving within 14 months.
Group 6: 2 milkings daily; in milk < 10 months; calving within 14 months.

[b] Computed from $\bar{f}$, V_f, V_M and r_{fM} by use of the formulas in table 1.

In some previous work (unpublished) on Register of Merit records of the Milking Shorthorn breed r_{fM}, r_{fF}, and r_{fFCM} (also $\bar{f}$, V_f and V_M) have been computed directly from the observations. These data are presented in table 2, together with r_{fF} and r_{fFCM} now computed by use of the present formulas. The values of the r's directly and indirectly computed agree very closely, affording a working check on the validity of the formulas.

DISCUSSION

Table 1 deals with purely algebraic relations and statistical methods. In table 2 these methods have been applied to certain biological material, namely, 6311 lactation or partial lactation records of individual cows with respect to per cent of fat (f) in the milk yielded, the amount of milk yielded (M), the amount of milk fat yielded (F), and the amount of milk energy yielded (FCM). We might deal further with the amount of milk sugar yielded (which would be roughly a multiple of M) or with the amount of milk protein yielded (which would be roughly a multiple of FCM). And, we might deal further with the per cent of sugar, the per cent of protein, the

per cent of water, in the milk yielded. That is, the possible considerations are several. The directly determined variables are f and M and we may with propriety deal with them first, but from a biological point of view we are concerned with the whole product of lactation, or that attribute of lactation which best represents the lactation metabolism of the cow.

Of all the 21 correlation coefficients given in table 2 all of the 7 r_{fM}'s, all of the 7 r_{fF}'s and none of the 7 r_{fFCM}'s are statistically significant at the one per cent level. This same condition, or close approach to it, prevails generally in the milk and fat records of dairy cows. The generalization may be drawn that a characteristic feature of lactation in dairy cows, as they are developed and managed in commercial milk production practice is, that, as between individual cows, milk-energy yield varies independently of per cent of fat in the milk. The same statement cannot be made with respect to milk yield or with respect to milk-fat yield.

A tempting explanation of why such a condition exists is that lactation metabolism or the work of lactation is directly proportional to FCM, and not directly proportional to M or to F; that the amount of work in lactation, as between cows, tends to be a constant so far as related to f (but of course, highly variable as affected by individuality outside the characteristic f); consequently, $r_{fFCM} = 0$. This explanation is sometimes referred to as the constant energy theory.

Groups 1 and 2 in table 2 differ only, so far as the record shows, in that group 1 was milked 2 times daily and group 2 was milked 3 times daily. Group 1 has a mean 12-months FCM of 8,292 (=7,731 calories of milk energy per day). Group 2 has a mean 12-months FCM of 11,351 (=10,573 calories of milk energy per day). This is an increase for 3 milkings over 2 of 37 per cent,[3] yet in both groups the constant energy theory holds good, that is, r_{fFCM} does not depart from 0 to a statistically significant degree. Under conditions of 2 milkings daily, lactation metabolism is at a certain level; under

TABLE 3

Values of r_{fM}, r_{fF}, r_{fFCM}, r_{MF}, r_{MFCM}, and r_{FFCM} at various values of V_f and V_M where $\bar{f} = 4$ and $r_{fFCM} = 0$

V_f	V_M	r_{fM}	r_{fF}	r_{fFCM}	r_{MF}	r_{MFCM}	r_{FFCM}
.1	.25	− .24	+ .16	0	+ .92	+.97	+.98
.1	.1	− .60	+ .45	0	+ .45	+.81	+.90
.2	.2	− .60	+ .45	0	+ .45	+.80	+.89
.1	.06	−1.00	+1.00	0/0[a]	−1.00	0/0[a]	0/0[a]

[a] That is, $\sigma_{FCM} = 0$, which condition, of course, will never turn up in any natural population. It should be clear that the expression, "constant" energy theory, does not imply that $\sigma_{FCM} = 0$, but only that FCM is "constant" so far as affected by differences in f, as between cows.

[3] If "age" is eliminated the increase is 39 per cent. The Bureau of Dairy Industry makes a "correction" for 3 milkings based on an increase of 24 per cent; the Holstein-Friesian Association, similarly, of 25 per cent.

conditions of 3 milkings daily, lactation metabolism is at a 37 per cent higher level. Yet, under either one of these conditions the amount of lactation metabolism (FCM), as between cows, is independent of fat per cent (f), or composition of the milk. The relation, $r_{fFCM} = 0$, must have a deep physiological significance.

On the theory that $r_{fFCM} = 0$ is a general phenomenon of lactation among dairy cows, table 3 has been prepared from table 1. For simplicity it is taken that $\bar{f} = 4$. The first line may be taken as typical of the correlations as found within a 4-per cent breed of dairy cows. The second line represents what would be found if V_M were reduced to the level of V_f, say by dealing with the average of several suitable partial lactations of individual cows, all of the same initial live weight and all under the same environment. The third line represents what would be found by including dairy cattle in general,[4] but with V_M reduced as in the second line. The last line represents the upper limit of V_f/V_M which can exist at $\bar{f} = 4$ and still have $r_{fFCM} = 0$, according to table 1.

In the first line of table 3 the high values of r_{MF}, r_{MFCM} and r_{FFCM} are striking. They are quite natural in the sense that M, F, and FCM are all more-or-less good measures of the level of lactation metabolism and must agree with each other to a great extent. As measures of lactation metabolism, M, F, and FCM are listed in order of increasing goodness, in the sense of direct proportionality to the amount of lactation metabolism. Within a breed, under usual conditions, dealing with M, F, or FCM will result in pretty much the same statistical outcome. Apparently, this outcome might be somewhat different under the unusual conditions represented by the other lines of table 3. Inasmuch as these have not been encountered experimentally, (however, *cf.* 1, footnote 4) the data are presented primarily for such philosophical consideration as they may be worth.

SUMMARY

Formulas are derived expressing the coefficient of correlation (r) between any two of the variables, fat per cent (f), milk yield (M), milk-fat yield (F) and milk-energy yield (FCM), entirely in terms of f and M. The 4 statistics, $\bar{f}$ (= mean f), V_f ($= \sigma_f/\bar{f}$), V_M and r_{fM}, computed from the observations of f and M afford the only data needed for the computation of r_{fF}, r_{fFCM}, r_{MF}, r_{MFCM} and r_{FFCM}, by use of the formulas. It is presupposed that n is large and the frequency distributions normal, or fairly so.

A previous result is confirmed: if $r_{fFCM} = 0$ (as is characteristically the case among dairy cows) it follows as an algebraic corollary that $-r_{fM} = \frac{\bar{f}}{8/3 + \bar{f}} \frac{V_f}{V_M}$. Biologically the relation, $r_{fFCM} = 0$, is regarded as primary, and

[4] In dairy cattle as a species it is likely that V_f is in the neighborhood of .2. In mammals as an order V_f may be in the neighborhood of .3 or .4.

interpreted to mean that the amount of lactation metabolism is proportional to FCM and is a common denominator as between cows differing in f, but otherwise comparable.

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THE DETERMINATION OF FAT IN THE PRESENCE OF FREE FATTY ACIDS. I. THE MOJONNIER TEST OF MIXTURES OF FREE FATTY ACIDS AND BUTTERFAT

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It has been the experience of some Mojonnier testers that rancid* dairy products give lower fat tests than expected. This may be explained by assuming that the fatty acids liberated from butterfat by lipase action are retained in the water layer as ammonium soaps. Data on the magnitude of this decrease, as well as correlation of the decrease with the degree of rancidity, would be of value in the study of lipase activity. Such data might answer several questions: 1. Is all of the free fatty acid extracted by the ammoniacal layer? 2. If not, is a constant fraction recovered in the ether layer? 3. Does the distribution depend on the nature of the acid?

These questions might be answered by Mojonnier fat tests on samples containing known quantities of neutral butterfat and of free fatty acids. If one assumes that lipase is non-specific, *i.e.*, attacks all triglycerides with equal facility, it should be possible to prepare a mixture which would approximate the composition of completely lipolyzed butterfat. This synthetic "rancid butterfat" should contain fatty acids in the proportions usually found in a typical butterfat. Addition of varying quantities of such a mixture to samples of butterfat, would yield products resembling butterfats of varying degrees of rancidity. Data obtained by subjecting such samples to the Mojonnier fat test might be useful in interpreting the phenomenon of fat loss in rancid dairy products.

EXPERIMENTAL

The average fatty acid composition of typical butterfats has been reported by a number of workers (*cf.* the bibliography of Davies (2)). A mixture of fatty acids was prepared using the ratios given by Davies (2). The relative amounts listed by Davies and the actual quantities used in the mixture are shown in table 1.

It should be noted that certain of the acids were not available and, for this reason, do not appear in the mixture. The mixed fatty acids were melted at 70° C. and the mixture was stirred continuously until it had solidified at room temperature.

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* Rancidity in these papers is limited to hydrolytic deterioration of fats.

TABLE 1

A comparison of the fatty acid distribution in a typical butterfat with the composition of the mixture of fatty acids used in these experiments

Name of acid	Distribution (after Davies)	Mixture used
	%	%
Butyric	3	3.6
Caproic	2	2.1
Caprylic	1	0.0
Capric	2	0.0
Laurie	4	4.4
Myristic	11	11.6
Palmitic	28	29.5
Stearic	9	10.5
Oleic	34	38.2
Linoleic	4	0.0

Quantities of a butter oil which had been prepared from a fresh, high-score, sweet-cream butter and which contained 98.53 per cent of Mojonnier-extractable fat were weighed into Mojonnier flasks. To the same flasks were added weighed amounts of the fatty acid mixture. The samples were then subjected to the conventional extraction procedure which is recommended by Mojonnier and Troy (4) for butter. The relevant data on these analyses are presented in table 2.

TABLE 2

The relationship between the amount of free acid in a sample of fat and the weight of material recovered by the Mojonnier test

Sample number	A Fat. Weight of Mojonnier extractable fat in butter-oil added	B Free fatty acid. Weight of fatty acid mixture added	C Sample. Fat plus free fatty acid (A + B)	D Recovered at end of extraction	E Fatty acid not recovered (C − D)	F Per cent of sample not recovered. $\frac{E \times 100}{C}$	G Per cent of fatty acid not recovered. $\frac{E \times 100}{B}$
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	%	%
1	401.0	0.0	401.0	401.0	.....	0.00	.....
2	362.3	3.7	366.0	363.1	2.9	0.79	78
3	360.4	14.3	374.7	364.8	9.9	2.64	69
4	312.3	30.2	342.5	320.1	22.4	6.54	74
5	286.7	98.2	384.9	309.8	75.1	19.51	76
6	196.2	219.5	415.7	245.6	170.1	40.92	77
7	97.3	294.4	391.7	163.8	227.9	58.18	77
8	0.0	412.8	412.8	86.0	326.8	79.17	79

DISCUSSION

An examination of these data indicates that a part of the free fatty acids is recovered by the Mojonnier test, but a larger part is lost in the ammoniacal layer. A comparison of the quantity of sample not recovered (E) with the quantity of fatty acid initially present in the sample (B) reveals a direct

correlation. If these absolute losses in fat test are calculated as percentages of the total free fatty acids present in the samples, a tolerably constant value (G) averaging 76 per cent is obtained. This means that 76 per cent of the particular mixture of free fatty acids which were used in these experiments was not extracted in the Mojonnier fat test. Since the quantity of free fatty acid in an actual sample is usually not known, and varies depending on the degree and quality of rancidity (lipolysis), the Mojonnier fat test on rancid samples will be decreased by a variable, unpredictable quantity.

That such decreases may be significant in market milk has been demonstrated by the work of Holland (3) who reported that composite samples of raw milk showed a progressive decrease in Mojonnier fat test which reached 0.3 per cent after 14 days' storage. Since composite samples of pasteurized milk showed no comparable decrease, he attributed this loss to lipase activity in the raw milk samples, with the consequent liberation of fatty acids which were not extracted by the Mojonnier procedure.

The magnitude of this decrease when testing butter is indicated by Bird and Breazeale (1) who reported that rancid butter gives an appreciably lower fat test by the Mojonnier method than by the A.O.A.C. official method. In certain cases this difference amounted to three or four per cent of butterfat. Considerably smaller differences were found when fresh butter was examined. Bird and Breazeale (1) believed that these results were obtained because the A.O.A.C. method determines fat plus free fatty acid, while the Mojonnier determination gives a fat test equal approximately to fat alone.

Our results substantiate this belief and indicate that the Mojonnier fat test includes all the neutral butterfat plus a fraction of the free fatty acids which might be released by lipase activity in a rancid sample. Experiments which will be published later demonstrate that the failure of the Mojonnier method to recover the individual free fatty acids of lipolyzed butterfat varies in degree with the different acids.

SUMMARY

Known weights of a mixture of fatty acids approximating completely hydrolyzed butterfat were added to known weights of butterfat. When these mixtures were tested by the Mojonnier method, a part but not all of the free acid was recovered along with the fat. The amount of free acid recovered with the fat was a practically constant fraction of the total free acid present in the sample. In the case of this particular mixture of acids, 24 per cent was recovered with the fat.

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RELATIONSHIP OF CURING TEMPERATURES TO QUALITY OF AMERICAN CHEDDAR CHEESE

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In the past 30 years the practice of storing American Cheddar cheese at 34° F. has become almost universal. It is the usual custom to deliver cheese to the warehouse 4 or 5 days after it is taken from the press. At the warehouse it is stacked on the receiving floor, where the temperature may vary from 50° to 70° and remains there from 1 to 5 or 6 days, depending on the volume handled. The boxes from each factory, identified by a factory and vat number, are stacked together and the cheese is graded by plugging one from each lot. The cheese is then paraffined and placed in storage at 34° where it is held until it is sent to the trade.

The practice of storing at 34° F. is a radical change from the early custom of curing Cheddar cheese at much higher temperatures. Until the work of Babcock and Russell (1) in 1897, 50° was considered a very low temperature, and cheese was usually cured at 65° to 70° and even higher. These investigators state that when the scoring was done at an early age, the low-temperature cheese had not cured sufficiently to meet the demands of the market, while, if the comparison was delayed until the low-temperature cheese was properly cured, that at the high temperature was of inferior quality and greatly deteriorated.

Smith (2) in 1900, cured cheese at 80°, 75°, 70°, 65°, 60°, and 55° F., and found that on the average the cheese that was held at 60° and 55° scored about 5 points higher in flavor and 2½ points higher in texture than the cheese that was held at the higher temperatures. While cheese that was held at 60° and 55° showed a great improvement in quality and uniformity over cheese held at higher temperatures, there was no improvement at 55° over 60°. In fact, some cheese had a better flavor when cured at 60° than when cured at 55° and the texture was equal in quality.

Babcock and Russell (3) found that cheese held at from 35° to 40° F. was more uniform than cheese that was cured at 60° or higher. In general, the cheese that was held at the lower temperatures developed a fine, silky body and close texture, the flavor was clean but very mild, and sharp flavors did not occur. They stated that the development of off-flavors during the curing period is partly caused by the use of tainted milk or faulty manufacture and concluded that holding at the lower temperatures is desirable because it results in a mild flavored cheese which is more preferred by the general trade than a cheese with a sharp flavor.

Received for publication October 10, 1940.

Van Slyke, Smith, and Hart (4) reported that in a comparison of curing at 40°, 50°, and 60° F., the best cheese was obtained at 40° and the cheese that was cured at 50° was superior to that cured at 60°.

Babcock, Russell, Baer, Van Slyke, Smith, and Hart (5) found that at the end of 5 months cheese was more uniform in quality when cured at 40° F. than when cured at 50° or 60°. Firm, typical Cheddar, when cured at 60° was of excellent quality and developed faster than cheese held at low temperatures, but in time the latter cheese was superior to that cured at 60°.

Doane (6), while agreeing in general with the earlier work on "curing" at low temperatures, pointed out the relation of the initial quality of the cheese to the effect of the curing temperature. In some cases his experimental cheese was of inferior quality when cured at high temperatures while identical cheese held at 32° F. was good. On the other hand, some lots of cheese scored higher when cured at 40° than identical cheese held at 32°. These particular lots received a good score at all temperatures, even at the variable temperatures of factory curing rooms. Cheese in which excessive acid was developed in the making did not show an effect of this acid when moved from the press for immediate storage at 32°, but if cured at higher temperatures the score was materially reduced by acid defects.

Dahle and Freeman (7) stated that the maximum flavor is reached more quickly if the cheese is cured at 63° F. than if it is cured at 45° and that the flavor score of the cheese cured at 63° is as high as that cured at 45°.

With the exception of the work of Doane, no attempt has been made to relate the initial quality of the cheese to the effect of the curing temperature on the flavor at the time it reaches the consumer, but it is evident that cheese properly made from good quality milk may be cured at temperatures as high as 60° F. and that a satisfactory flavor may be obtained. On the other hand, Doane has pointed out that the unfavorable effects of defective manufacturing methods which become very evident in cheese cured at high temperatures are suppressed if the cheese is held at 32° F.

The storage temperature generally adopted by the industry (34° F.) is lower than that recommended by the Bureau of Dairy Industry and has resulted in a cheese which, while it may be free from abnormal flavors, is almost entirely lacking in the pleasing, characteristic flavor of the English Cheddar cheese.

It is generally considered that the average consumer prefers a mild-flavored cheese. This demand is met by marketing cheese that has been held for a relatively short period at 34° F., which retards curing or the development of flavor. However, there are many consumers who prefer a cheese with a distinct, and even a sharp, flavor, but who are seldom able to find such cheese on the market.

It seems very probable that the consumption of cheese would be increased, and the industry benefited correspondingly, if cheese were made so that it

could be cured at temperatures high enough to permit the development of a characteristic Cheddar flavor.

The experiments reported in this paper were planned to determine the relationship of various curing temperatures to the quality of American Cheddar cheese.

METHODS

The milk used was produced on the Bureau's farm at Beltsville, Maryland, and was of exceptionally fine quality, high in solids, and low in bacteria count. It had a methylene blue reduction time of over 5 hours. It was delivered to the laboratories at 40° F., where it was made into cheese without being pasteurized.

In making the cheese for this experiment a uniform standardized method, which was designed to make good cheese, was followed about half the time; the rest of the time the method varied in the rate of acid development and the amount developed, the time the curd was allowd to lie in the whey, and in the moisture content introduced in the cheese. This procedure was followed in order to simulate the variety of manufacturing conditions (good and indifferent) under which cheese is usually made and stored in commercial practice.

Altogether 122 vats of milk were made into cheese and packed in the form of daisies. The 4 daisies from each vat were placed in storage and held as follows:

One was held 6 months at 34° F. to simulate the usual storage practice. This may be considered the "control" cheese.

One was held 3 months at 50° F., plus 3 months at 34°.

One was held 6 months at 50° F.

One was held 3 months at 60° F., plus 3 months at 34°.

The temperatures were maintained within a variation of $\pm$ 1° and the relative humidity was 70 to 75 per cent.

The cheeses were scored when 3 months old and again at 6 months. The scoring at 3 months gave an indication of the final quality, but the tabulations below are based on the scoring at 6 months.

EXPERIMENTAL RESULTS

Relation of storage time and temperature to quality. By the end of the experimental period, 116 sets of 4 cheeses each were available for comparison. The 3 cheeses of each set that were cured under 3 different experimental procedures were compared with the fourth (control) cheese of the same set, which was stored 6 months at 34° F.

Table 1 shows the number of cheeses that scored higher or lower than the control cheese, and the points of difference in each case. All scoring was done after a storage period of 6 months.

TABLE 1

Quality of cheese cured at various temperatures as compared with the quality of the control cheese (held 6 months at 34° F.)

Number of cheeses, experimental, storage period, and temperature	Cheeses higher in score than the control								Cheeses equal in score to the control		Cheeses lower in score than the control							
	Higher by						Total higher				Lower by						Total lower	
	3 points	2½ points	2 points	1½ points	1 point	½ point					½ point	1 point	1½ points	2 points	2½ points	3 points		
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*%*	*No.*	*%*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*%*
116 cheeses: 3 months at 50 and 3 months at 34° F.	.....	.....	5	5	26	31	67	57.7	17	14.7	20	4	6	2	.....	.....	32	27.6
116 cheeses: 6 months at 50° F.	.....	1	1	9	21	29	61	52.5	15	13.0	20	10	6	2	2	.....	40	34.5
116 cheeses: 3 months at 60 and 3 months at 34° F.	.....	1	3	9	19	16	48	41.4	14	12.0	18	13	13	4	1	5	54	46.5

It will be noted that 57.7 per cent of the cheeses that were cured 3 months at 50° plus 3 months at 34° had a higher score than the control cheese and that 14.7 per cent had the same score as the control; also that 52.5 per cent of the cheeses that were cured 6 months at 50° had a higher score than the control and 13 per cent had the same score as the control. On the other hand, only 41.4 per cent of the cheeses cured 3 months at 60° plus 3 months at 34° had a higher score than the control.

In this tabulation (table 1) no distinction was made between the cheese that was properly made and the cheese which could be expected to develop defects and undesirable flavors because of faulty methods of manufacture. Since all the cheese was made from milk which, by all the accepted standards, was of excellent quality no defect in the cheese could be ascribed to the milk.

There was, however, an even greater variation in the quality of the cheese than is indicated in table 1. The range in score for all the cheeses was from 87 to 95, but in table 1 the comparison in each case was between 4 cheeses made from the same vat of milk, and no attempt was made to group the cheeses according to grade.

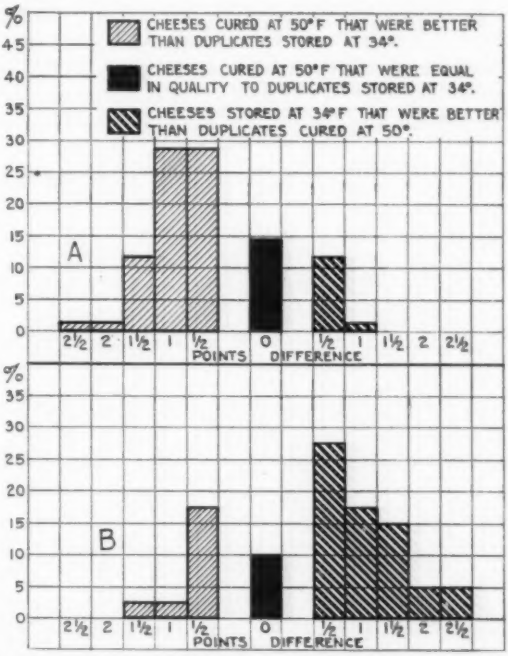

FIG. 1. Influence of storage at 34° F. and at 50° F. on the quality of duplicate pairs of cheese: A. Cheese that had a grade of 92 or righer; B. Cheese that had a grade below 92.

Figure 1 indicates the difference between the effect of storage temperature on high-scoring cheese and on low-scoring cheese. For this comparison, all the cheeses that had been cured 6 months at 50° F. were divided into two groups, one group including those that scored 92 or more, and the other group those that scored below 92.

In the group that scored 92 or more, 72.38 per cent scored from ½ to 2½ points higher than their duplicates that were stored 6 months at 34° F., and 14.47 per cent scored the same.

In the group that scored below 92, the results were almost the reverse. Of those held at 34° F., 70 per cent were better than the duplicates that were cured at 50°, and 10 per cent scored the same.

It should not be inferred that some of these cheeses had a tendency to develop a better flavor in storage at 34° than at the higher temperatures. The correct explanation is that when cheese is so manipulated in the making that it has a definite tendency for defects in flavor or texture, these defects develop promptly if such cheese is held at temperatures high enough to permit these factors to act; while if such cheese is held at 34° F. the unfavorable activity is suppressed. On the other hand, cheese properly made does not develop flavor when stored at 34° F. but it does develop a characteristic Cheddar flavor in a reasonable time if cured at 50° F. Past observations have also shown that when milk of inferior quality is used, defects are less pronounced when the cheese is held at 34° F. than at higher temperatures.

Relation of moisture content to quality. Moisture confent is one of the factors that may be expected to have an influence on the final quality of Cheddar cheese. In the cheeses used in this experiment, moisture content varied from a minimum of 32.28 per cent to a maximum of 40.97 per cent. Of the total number of cheeses 11.2 per cent had less than 36 per cent of moisture; 58 per cent had 36 to 37.92 per cent of moisture; 30.6 per cent had 38 per cent of moisture or more, but only 12 per cent had 39 per cent of moisture or more.

It is well known that a high-moisture cheese is likely to develop a sharp flavor, and that it will ripen faster than a low-moisture cheese. The cheeses in this experiment were divided into two groups, one having a moisture content of 38 per cent or higher, the other having less than 38 per cent of moisture. Figure 2 shows the percentage of cheese in each moisture group that scored 92 or higher, for the four storage procedures used in this experiment.

A moisture content of 38 per cent or higher did not affect appreciably the quality of the cheese stored at 34° F. When the cheese was held 3 months at 50° plus 3 months at 34° the cheese with a moisture content below 38 per cent was of distinctly higher grade than that containing 38 per cent or more and at the higher temperatures the detrimental effect of the high moisture

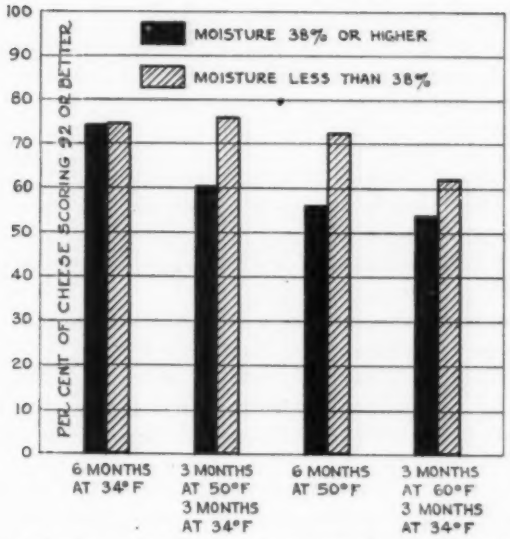

FIG. 2. The influence of the moisture percentage on the grades of cheese stored at different temperatures.

was even more evident. Under all conditions except continuous storage at 34°, the low-moisture group had a higher percentage of cheese scoring 92 or better. In other words, the unfavorable influence of the high-moisture content was suppressed at 34° F., but was active at the higher temperatures.

Some of the high-moisture cheese cured satisfactorily and developed a clean desirable flavor at all temperatures, indicating that the high moisture in itself is not necessarily a cause of objectionable flavors.

We believe that cheese properly made from milk of good quality will cure satisfactorily even if the moisture content is slightly higher than 38 per cent.

Relation of quality of milk to quality of cheese. Observations on cheese made in a commercial factory with records on the making process and on the grades of the cheese at 3 months give some indication of the influence of the quality of the milk on the grade of the cheese. Cheese was made from 57 vats of milk, 76 per cent of which reduced methylene blue in less than 2 hours, and 24 per cent in 1 hour or less. Duplicate cheeses from each vat were stored at 34° and at 60° F. and graded when they were about 3 months old.

Of the cheese stored at 34°, 17.3 per cent scored 92 or better; but of those stored at 60°, only 1.3 per cent scored as high as 92. These data indicate that, when a dealer is storing cheese about which he has little knowledge, beyond what may be obtained by the examination of a plug, the only safe procedure is to follow the present custom and store at 34° F. On the other

hand, it is evident that cheese may be made which can be cured at temperatures as high as 50° F. to develop the desirable and characteristic Cheddar cheese flavor, by controlling the quality of the milk and the manufacturing methods.

SUMMARY

One hundred and sixteen vats of milk of good quality were made into cheese, and the four duplicate cheeses made from each vat were stored as follows: One was held for 6 months at 34° F.; one for 3 months at 50° plus 3 months at 34°; one for 6 months at 50°; and one for 3 months at 60° plus 3 months at 34°.

In the group stored for 3 months at 50° plus 3 months at 34° F., and also in the group stored 6 months at 50°, the majority of the cheeses were better in quality than the duplicates (or controls) that were stored 6 months at 34°. But in the group stored 3 months at 60° plus 3 months at 34°, the majority of the cheeses were poorer in quality than the duplicates stored 6 months at 34°.

Of the 116 cheeses that were stored 6 months at 50° F., about 68 per cent had a score of 92 or more at the end of the storage period and the other 32 per cent had a score of less than 92. Of the high-scoring group, 87 per cent were better in quality than, or as good as, the duplicate that was stored 6 months at 34°; whereas, of the lower-scoring group, only 32 per cent were better than, or as good as, the duplicate stored 6 months at 34°.

From these data, it appears that if a cheese is destined as a result of some inherent defect in the making process to have a score below 92, it will have a relatively higher score if it is held at 34° F. than if it is cured at 50°.

When cheese is held 6 months at 34° F., the percentage that will score 92 or better will be about the same whether the moisture content is above or below 38 per cent. When cheese is cured at any of the three higher temperatures used in this experiment, however, the percentage scoring 92 or better will be from 5 to 15 per cent in favor of the low-moisture cheese.

Cheese made from milk of good quality and by methods which insure cheese of good quality can be cured at temperatures as high as 50° F. with reasonable certainty of developing a clean and characteristic Cheddar flavor.

From past observations cheese made from bacteriologically poor milk or so manipulated that there is a tendency to develop acid, bitter, or other off-flavors, should be stored at 34° F. in order to retard the development of these defects as much as possible.

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American Dairy Science Association Announcements

THIRTY–SIXTH ANNUAL MEETING, UNIVERSITY OF VERMONT, BURLINGTON, VERMONT, JUNE 23–26, 1941

Call for Titles and Abstracts

See the January issue of our JOURNAL for the regulations.

"The titles and abstracts must be in the hands of the program committee **not later than April 15,** 1941. This committee consists of Glen W. Vergeront, University of Wisconsin, chairman of the program committee of the Extension Section; Walter V. Price, University of Wisconsin, chairman of the program committee of the Manufacturing Section; W. E. Petersen, University of Minnesota, chairman of the program committee of the Production Section; and E. S. Guthrie, Cornell University, general chairman. The titles and abstracts should be sent to the sectional chairman concerned."

PRESIDENT'S MESSAGE

TO THE MEMBERS OF THE AMERICAN DAIRY SCIENCE ASSOCIATION:

Our association will soon be completing its thirty-fifth year of existence. Its history is a record of steady growth and increasing influence in a great industry. I am sure that we can all be extremely proud of the preeminent position which it now holds. However there are still unlimited possibilities for further expansion.

Membership in the association has increased from a mere handful of dairy instructors at the beginning to nearly 1500 workers throughout the industry at the present time. Each month over 2400 copies of the JOURNAL OF DAIRY SCIENCE are mailed to subscribers. Such a wide dissemination of late scientific information must have an important influence in directing the work and thought of those associated with the dairy industry.

Recent important steps have been taken to make the JOURNAL of even greater value than in the past. The abstracting service has been revised in such a manner as to make it more complete and of improved quality. Due to the large number of papers presented to the JOURNAL OF DAIRY SCIENCE and the fact that they are published in the order of their acceptance, a given paper may not appear in print for several months after being submitted. This objection is being met by printing soon after the acceptance of a paper, an advance abstract which will give the work reported its desired priority.

An index covering the first 20 volumes of the JOURNAL is well along in preparation. Back numbers of the JOURNAL which were out of print have been reproduced so that complete back volumes are now available.

While the JOURNAL OF DAIRY SCIENCE is an important phase of the work of the association it is not by any means its sole activity. Scores of our members are working diligently on committees to improve the work of the association and increase its scope of influence. One large committee is studying dairy curricula, the many questions of which our association should logically take the lead in answering. According to present plans a discussion of dairy curricula will have an important place on the program of the next annual meeting of our association. Other committees are working on standard methods to be recommended for use in bacteriological and chemical analyses of dairy products and also methods for feed, feces, vitamin, enzyme, endocrine, blood and urine analysis. Committees on Breed Relations, Feed Specifications, Sanitary Procedure and Quality Improvement and many others are working to help solve the problems of the industry.

Already the dairy industry is looking to the association for help in the standardization of many practices. Score cards used in scoring dairy products or dairy cattle, are those approved by the American Dairy Science Association. Rules for conducting students national contests in judging dairy cattle and dairy products and the general sponsorship of the contests are in the hands of our association. The dairy breed associations are now using methods of testing for production and for reporting production tests which in many cases have been recommended by our association and there is little doubt that much unification of the testing methods of the future can be accomplished through our committees which are constantly considering these problems.

One important activity of the American Dairy Science Association is the administration of the Borden Awards for outstanding research in the fields of dairy manufacturing and dairy production.

Due to the growth of the association and the diverse interests of its members the planning of a program for the annual meeting has become a real problem. There is inadequate time for the presentation of all the papers submitted. The present program committee proposes to solve this difficulty by the planning of symposia and the dividing of sectional programs into several sessions running simultaneously.

This year the summer meeting of the association is to be held at Burlington, Vermont, on June 23rd to 26th. This should provide an excuse for a most interesting and worth while trip for a large group of our members.

Sincerely yours,
(Signed) H. W. CAVE,
President

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ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS ACCEPTED FOR PUBLICATION IN THE JOURNAL OF DAIRY SCIENCE

47. The Freezing and Thawing of Milk Homogenized at Various Pressures. G. M. TROUT, Michigan Agricultural Experiment Station, E. Lansing, Mich.

Analyses of various portions of milk homogenized at different pressures and frozen partially or wholly have been made.

When creaming was inhibited by heating or by homogenization the unfrozen portion was relatively richer in fat and solids-not-fat than the frozen portion. When creaming occurred, as in the unhomogenized milk, the frozen portion was higher in fat but lower in solids-not-fat than the unfrozen portion.

The titratable acidity of the unfrozen portion was higher than the frozen portion regardless of creaming. No evidence of creaming was noted in frozen homogenized milk but rather a pronounced settling of the fat which was influenced by the rate of thawing. However, creaming occurred to some extent when milk heated to inhibit creaming was frozen.

Frozen homogenized milk upon thawing exhibited no flakiness which was commonly observed when unhomogenized milk was frozen and thawed, but did exhibit a watery appearance at the surface layers which was more pronounced when the frozen milk was thawed slowly.

Marked settling of the solids of milk was noted when homogenized milk was frozen and then thawed. The rate of thawing had a pronounced influence upon the extent of settling of the fat and solids-not-fat; the lower 15 per cent of creaming cylinders of slowly thawed frozen homogenized milk contained as high as 7.7 per cent fat and 24.60 per cent total solids as contrasted to 2.0 and 5.50 per cent respectively of the upper 15 per cent layer. The titratable acidity of the upper and lower 15 per cent portions ranged from 0.04 to 0.35 per cent. Similar trends were observed upon freezing and thawing of skimmed milk.

The drainage of solids from homogenized milk was slightly faster after the first 100 ml. than that of the unhomogenized milk.

48. Thermoduric Bacteria in Pasteurized Milk. II. Studies on the Bacteria Surviving Pasteurization, with Special Reference to High-Temperature, Short-Time Pasteurization. J. L. HILEMAN, HENRY LEBER, AND M. L. SPECK, Dairymen's League, Syracuse, N. Y.

Studies in a single large pasteurizing plant with about 400 producers showed that the pasteurization of milk in commercial equipment at 161° F. for 16 seconds resulted in higher bacteria counts than pasteurization of the

same milk in the laboratory at 143–144° F. for 35 minutes. The higher counts in the milk pasteurized by the high-temperature method were largely due to the ability of certain species of micrococci to survive in larger numbers in milk pasteurized by that method than in milk pasteurized by the low-temperature long-hold method. The commonest species of micrococci among those found in the milk pasteurized at the higher temperature were *M. candidus, M. epidermidis, M. luteus* and *M. varians,* although five other species were encountered less frequently. These micrococci make up the predominant thermoduric flora of dirty milking machines, strainers and pails on farms.

The commonest species of streptococci encountered were *S. bovis* and *S. thermophilis.*

49. Oxidized Flavor in Milk. VIII. The Effect of the Degree of Saturation of Fat in the Ration of the Cow upon the Iodine Number of the Butter Fat and the Susceptibility of the Milk to Metal-Induced Oxidized Flavor. W. CARSON BROWN, R. B. DUSTMAN, AND CHAS. E. WEAKLEY, JR.

In a feeding experiment, eleven cows, whose milks were susceptible to metal-induced oxidized flavor, were selected and placed on a ration low in fat. Samples of milk were collected from each cow each morning and pasteurized in bottles. The milk was then divided into two lots, one of which was contaminated with copper and stored for three days after which it was judged for flavor. The other lot was composited by individuals and churned. The duration of the experiment was divided into four periods, viz.: 1. Preliminary period of six weeks in which the normal variations were observed; 2. A feeding period of six weeks in which saturated and unsaturated vegetable oils were fed; 3. A reversal period of four weeks in which the chemical nature of the oil being fed was changed, and 4. A readjustment period of four weeks during which no supplementary oil was fed. The oils fed were, cocoanut oil (iodine number 11), expeller soybean oil (iodine number 127), crude soybean oil (iodine number 127), refined soybean oil (iodine number 127) and hydrogenated soybean oil (iodine number 17).

The following results were obtained: 1. The feeding of 1 pound of cocoanut oil per animal per day decreased slightly the iodine number of the resulting butter fat and reduced slightly the intensity of the oxidized flavor; 2. The feeding of 1 pound of expeller or crude or refined soybean oil increased greatly the iodine number of the resulting butter fat and increased the susceptibility of the milk to oxidized flavor; 3. The feeding of 1 pound of hydrogenated soybean oil increased slightly the iodine number of the resulting butter fat. This was believed to be due to the desaturation of the fat by the cow; 4. The difference in the phospholipoid (lecithin) content of the different types of soybean oils produced no noticeable change in the

development of oxidized flavor; 5. In a ration low in fat the change in iodine number of the butter fat does appear to be correlated with the intensity of oxidized flavor. It is believed that other factors are of more importance; 6. The iodine numbers of the butter fat ranged from 23 for the cocoanut oil supplement to 58 for the expeller oil supplement; 7. Under the conditions of this experiment approximately three weeks were required before the iodine number of the butter fat had reached the period of maximum change.

50. Variations in Bull Semen and Their Relation to Fertility. ERIC W. SWANSON AND H. A. HERMAN, Department of Dairy Husbandry, University of Missouri.

A study of the characteristics of 256 samples of dairy bull semen, collected from ten sires of known breeding efficiency, and their relation to fertility is reported. Factors studied included volume of ejaculate, concentration of spermatozoa, pH, motility, viability under storage conditions, and sperm abnormalities.

Wide variations in all properties of semen were observed in successive ejaculates from the same bull, as well as ejaculates from different bulls. No single factor was found highly indicative of the fertility of fresh semen from these sires, which averaged from 1 to 3.67 services per conception. However, the factor found to be most indicative of a bull's fertility was the length of time vigorous motility was maintained in stored semen. The viability of any one sample of semen from a fertile bull could be forecast roughly from the type of initial motility present.

The average pH, volume, concentration, initial motility, and percentage of abnormal spermatozoa were correlated with a bull's fertility only insofar as they agreed with the time of survival of vigorous motility.

51. The Use of Nembutal Anesthesia in Milk Secretion Studies. E. P. REINEKE, M. B. WILLIAMSON, AND C. W. TURNER, Missouri Agricultural Experiment Station.

It was shown that goats maintained under nembutal anesthesia continue to secrete milk of normal composition at the normal rate. Comparisons between arterial and mammary venous blood samples drawn from goats anesthetized with nembutal showed that the uptake of milk precursors continues under these conditions. In view of these results it was pointed out that the adverse effects caused by excitement of the animal while drawing arterial and venous blood samples may be eliminated by the administration of nembutal.

52. Mastitis. I. The Relationship of the Development of Mastitis to Changes in the Chlorine, Lactose, and Casein Number of Milk.

A. H. VanLandingham, Chas. E. Weakley, Jr., E. N. Moore, and H. O. Henderson, West Virginia Agricultural Experiment Station, Morgantown, W. Va.

A study was made of the chlorine, lactose, chlorine-lactose number and casein number of the fore-milk from individual quarters in normal udders and udders with mastitis.

The fore-milk from normal quarters was found to contain an average of 0.124 per cent chlorine, and 4.79 per cent lactose. The average chlorine-lactose number was 2.61 and the casein number 77.6. The mean difference between quarters of normal udders was chlorine 0.007 per cent, lactose 0.115 per cent, chlorine-lactose number 0.184 and casein number 1.00.

Diagnosis of mastitis by changes in the above indirect biochemical tests based upon quarter differences practically eliminates difficulty due to changes in the chemical composition of the milk from time to time, as well as changes associated with advanced stages of lactation and the individuality of the animal.

In order to detect chronic mastitis, the quarter with the lowest percentage chlorine or chlorine-lactose number and the highest percentage lactose or casein number was considered as normal. For a significant difference between normal quarters and affected quarters there must be a difference of at least 0.02 per cent for chlorine, 0.36 per cent for lactose, 0.60 for chlorine-lactose number, and 2.90 for casein number.

Quarters may show bacteriological changes in the fore-milk indicating ineipient stages of chronic mastitis without showing significant changes in the chemical composition of the milk.

53. Adequacy of "Home Grown" Rations in Protein and Mineral Matter for Growth in Dairy Heifers. I. W. Rupel, G. Bohstedt, and E. B. Hart, Departments of Dairy Husbandry and Biochemistry, University of Wisconsin, Madison, Wis.

Six lots of heifers were fed during a period of 532 days to study the adequacy of protein and mineral matter in "home grown" rations. The nutritive ratio averaged 1:6.5 to 1:6.7 for all except the basal unsupplemented ration which was 1:10.7. Timothy hay and corn silage were the roughages used in the basal ration and for two of the lots receiving supplemental feeds. Alfalfa hay and corn silage formed the roughage portion of the ration for the three remaining lots.

Simple home grown rations proved to be adequate for growth in dairy heifers. Alfalfa hay, corn silage, and a mixture of equal parts corn and oats produced excellent gains. The rate of growth was not improved by the addition of bone meal or wheat bran.

Timothy hay, corn silage, and corn and oats furnished too little protein for optimum growth. The simple addition of protein from corn in the form

of corn gluten meal promoted as rapid growth as was obtained by using a mixture of greater variety including wheat bran, linseed meal, and corn gluten meal in addition to corn and oats.

BACTERIOLOGY

54. The Influence of Various Factors on the Fermentation End-Products of the Heterofermentative Lactobacilli. C. C. THIEL, National Institute for Research in Dairying, Univ. of Reading, England. J. Dairy Res., *11:* 136–144. 1940.

The influence of temperature, oxygen tension, yeast autolysate, and chalk on the production of by-products and the ratios of by-products formed to sugar utilized and lactic acid produced in milk by the lactobacilli, was investigated and results summarized as follows:

1. The total production of lactic acid is increased by the presence of chalk and "growth factors" but is not affected by temperature or oxygen tension. The ratio of lactic acid produced to sugar utilized is decreased at lower temperatures and increased by chalk but yeast autolysate and variation in oxygen tension give variable results.

2. The total production of acetic acid is increased by the presence of chalk, and "growth factors" and by anaerobic conditions. The ratios of acetic acid produced to sugar utilized and lactic acid formed are decreased by chalk and anaerobic conditions and there is a tendency for the ratios to be decreased by the presence of yeast and increased at lower temperatures.

3. The total production of alcohol is increased by anaerobiosis and also by the presence of chalk and yeast in the medium. The ratios of alcohol formed to sugar utilized and lactic acid produced is increased by anaerobic conditions, and chalk also tends to increase the relative amount of alcohol. Temperature and yeast autolysate have little effect.

4. Chalk is effective in maintaining the pH at a higher level during growth, but the influence of chalk on the ratios of by-products formed to sugar utilized is probably not entirely a pH effect since the addition of talc to the medium has an equally strong influence in altering the relative production of lactic acid and alcohol.

5. The Pasteur effect (suppressing alcoholic fermentation by respiration) is slight for the heterofermentative lactobacilli. S.T.C.

55. Demonstration of Rapid Production of Lactic Acid in Oral Cavity. ISAAC NEUWIRTH AND JULIUS A. KLOSTERMAN, Depts. of Pharmacology and Therapeutics and Bacteriology, New York Univ. Proc. Soc. Exp. Biol. and Med., *45:* 464. 1940.

Experiments were conducted which demonstrated that lactic acid was rapidly produced in the oral cavity of man and *in vitro* as the result of the action of oral microorganisms on certain carbohydrates. R.P.R.

56. Effects of Sulfanilamide and Sulfamethylthiazol on Experimental Brucella (var. *melitensis*) Infection in Mice. P. MORALES-OTERO AND A. POMALES-LEBRÓN, Dept. of Bacteriology, Univ. of Puerto Rico. Proc. Soc. Exp. Biol. and Med., *45:* 512. 1940.

Sulfamethylthiazol was found more effective than sulfanilamide in the treatment of experimental Brucella infection in mice. The proliferation of the organisms was seemingly only partially inhibited by the treatment with a tendency towards the establishment of a chronic infection. R.P.R.

BREEDING

57. A Study of Persistency in a Herd of Ayrshire Cows. G. PONTECORVO, Inst. of Anim. Genetics, Univ. of Edinburgh, Scotland. J. Dairy Res., *11:* 113–120. 1940.

The central portion of the lactation curve, lying between the peak of daily production and the rapid decline (drying off) was the object of investigations in an inbred Ayrshire herd. Coefficients of persistency were obtained by fitting exponential curves to this central portion. After eliminating all first lactations and all abnormal lactations (abortion, mastitis (when known), foot-and-mouth disease, etc.), a total of 163 lactations, were available from fifty-nine cows, of which forty-one had two or more records and eighteen only one. The month of calving accounted for about one-third of the variance of P. Of the remaining variance approximately one-half was attributed to differences among cows. No conclusion was reached as to the relative importance of heredity and environment in determining persistency. S.T.C.

58. Change of the Age of Puberty in Albino Rats by Selective Mating. CALVIN P. STONE AND R. G. BARKER, Dept. of Psychology, Stanford Univ. Proc. Soc. Exp. Biol. and Med., *44:* 48. 1940.

Twenty-five pairs of albino rats 6 months of age and known to be fertile were mated and allowed to produce one litter each. Sixty-two males and 82 females were reared from the 25 litters. The mean age of first estrus for the 82 females was 51 days and the mean age at first copulation by the males was 58 days. The male used from the parental generation for the early strain copulated at 44 days of age and the 11 females for the early strain were first in estrus at a mean age of 40 days. For the late strain 2 males were used, one copulating at 70 days of age, the other at 76 days. The mean age at first estrus for the 10 females of the late strain was 65 days. In the F_6 generation the mean age of puberty was as follows: for 166 early males, 48 days; for 128 early females, 43 days; for 80 late males, 61 days; and, for 89 late females, 57 days. For each sex the difference in means was statistically significant. It was concluded that the lowering of the mean age of

puberty in rat colonies during the past 30 years may have been due in part to selective mating of early pubescent stock. R.P.R.

BUTTER

59. Preparation and Care of Starters. MICHAEL MICHAELIAN, Chicago, Ill. Nat. Butter and Cheese J., *31:* 11, 15. 1940.

Common defects in starters are discussed. *Acid or sharp flavor* is attributed to either high or low incubation temperatures, conditions which disturb the proper balance of the two types of organisms in starter. A starter which is *flat or lacking in flavor* may be low in acidity or lacking in such compounds as volatile acids, carbon dioxide, acetylmethylcarbinol and diacetyl, all of which should be formed from citric acid during starter ripening. *Heated, malty or caramel flavor* may be caused by prolonged pasteurizing at high temperatures or by growth of undesirable organisms. *Metallic flavor* comes from worn out metal containers and over development of starter organisms. *Oxidized or cappy flavor* is caused by oxidative changes induced by defective metal containers or exposure to light. *Off flavors* are also caused by excessive acidity, faulty milk supply, or contamination of cultures. *Fast coagulation* of milk is caused by holding at high temperatures and by large inoculations. *Slow coagulation* may be caused by lack of distribution of culture, by bacteriophage, by frequent development of excessive acidity in cultures, by lack of cooling when starters have correct acid, or by prolonged holding at low temperatures. *Viscous or thick body* may be caused by high milk solids, high temperatures or prolonged holding during pasteurizing or excessive acid. *Low viscosity* may be caused by milk of inferior quality, low *pasteurizing temperatures* (150° F. or less) or high temperatures (212° F.), jarring starter during coagulation, or low acidity. *Lumpiness* is caused by high incubation temperature, low pasteurizing temperatures or lack of stirring. *Wheying off* may be due to high or low acidity, agitation during coagulation, or excessive agitation of starter. *Ropiness* is caused by some starter organisms. W.V.P.

60. A New Method for the Detection of Neutralized Cream, Including a Comparison with Other Methods. D. J. HANKINSON AND E. O. ANDERSON, Storrs Agr. Exp. Sta., Storrs, Conn. Bull. 234, July, 1939.

A detailed study is presented of a comparison of the present methods which may be used to detect neutralizers in cream with a new procedure developed by the authors. A protein and fat-free serum is obtained by means of the precipitating action of uranyl nitrate. The method is based chiefly on pH and buffer measurements employing the principle that an increase in lactic acid results in an increase in the buffer constituents of the

creams. The effect of the uranyl nitrate as a buffer substance in the filtrate can be neglected since a constant amount is added in all determinations. From the standpoint of both accuracy and time required for analyzing many samples, the new method was found to be the most practical for identifying neutralized cream. P.H.T.

CHEESE

61. An Objective Measure of the Consistency of Cheese Curd at the Pitching Point. G. W. SCOTT BLAIR AND F. M. V. COPPEN, Nat. Inst. for Res. in Dairying, Univ. of Reading, England. J. Dairy Res., *11:* 187–195. 1940.

A small cylindrical sieve (16 mesh) is filled with curd by drawing it through the stirred contents of the vat. The curd is inverted onto a disk 5 seconds after the sieve leaves the vat and reinverted after 20 seconds. The sieve with contents is weighed at 27 seconds. The curd is then again inverted onto a metal platform. The height of the cylinder is measured at 50 seconds. The property (W/h) is suggested as a measure of consistency. It was found to be highly correlated with moisture content. S.T.C.

62. Factors Affecting the Quality of Limburger Cheese Made from Pasteurized Milk. M. W. YALE, New York (Geneva) State Agr. Exp. Sta. Tech. Bull. 253. 1940.

With few exceptions, limburger cheese is manufactured in this country from raw milk. The use of pasteurized milk presents new problems to cheesemakers. Factors affecting the quality of limburger cheese made from milk heated to 145° F. (62.8° C.) have been studied.

Forty lots of limburger cheese comprising 172 1-pound cheeses were made at a limburger cheese factory by the manufacturing method commonly used with raw milk. Both partial and complete pasteurization of the milk markedly improved the quality of cheese made in July and August from poor quality milk but not that made in October from milk of better quality. Pasteurization of the milk at 145° F. for 30 minutes resulted in as good cheese as partial pasteurization using holding periods of 0, 10, 15, and 20 minutes.

Limburger cheese made from pasteurized milk had a milder flavor than that made from raw milk. Flavor development in the pasteurized milk cheese varied with individual lots and was usually most pronounced when starter was not added to milk. With some lots starter was not beneficial, while with others the addition of 0.05 or 0.10 per cent of lactic starter improved the grade of the cheese. Results obtained with starter hold true only for the one method of manufacture used in these studies. Experience by the industry indicates that special manufacturing methods may be desirable with pasteurized milk.

Pasteurization of milk for the manufacture of limburger cheese results in a product of uniformly good quality when the proper manufacturing method is followed. Author's Abstract.

63. A Note on a Simple and Accurate Viscosimetric Form of Rennet Test. C. W. KING AND E. M. MELVILLE, Chem. Dept., The West of Scotland Agr. College, Glasgow. J. Dairy Res., *11:* 184–186. 1940.

Improvements, largely with respect to excluding drafts and securing a better form of support, are suggested to the apparatus previously described (J. Dairy Res., *10:* 340–354. 1939). S.T.C.

CHEMISTRY

64. The Stability of Vegetable Oils. II. Apparatus for Determination of the Rate of Fading of Methylene Blue-Fat Systems. W. G. BICKFORD AND K. S. MARKLEY. U. S. Regional Soybean Indust. Products Lab., Urbana, Ill. Oil and Soap, *17:* 232. 1940.

From the work done on soybean oil this apparatus and method seem well adapted for the determination of the relative stability of edible oils. The apparatus and method is applicable for the determination of: 1. The relative induction period of fats and oils; 2. The relative rate at which a fat or oil is capable of consuming its dissolved oxygen under standardized conditions, and 3. The extent of the prior consumption of dissolved oxygen at the time the fat or oil is subjected to examination, or the latent capacity of a fat or oil to consume its residual oxygen.

The fat stability apparatus described employs the principle of methylene blue reduction in a fat-dye system exposed to filtered radiations of a tungsten filament lamp. The apparatus is designed and constructed in such a manner that certain variables, such as the fluctuation in light intensity, temperature, infrared radiations, and the limitations of visual observation are controlled or eliminated. V.C.S.

DISEASE

65. Effect of Gramicidin Suspended in Mineral Oil on Streptococci of Bovine Mastitis. R. B. LITTLE, R. J. DUBOS, AND R. D. HOTCHKISS, Rockefeller Institute for Medical Research. Proc. Soc. Exp. Biol. and Med., *45:* 462. 1940.

Of 31 quarters naturally infected with *Streptococci agalactiae* and treated with a gramicidin (120–160 mg. of bactericidal substance)-sterile mineral oil mixture, 26 appeared to have responded by a complete disappearance of the streptococci. In some of the cured cases the infection was of a severe chronic nature. A single treatment was sufficient in 20 of 31 quarters while

6 quarters required additional treatments. Of 4 cases of bovine mastitis caused by *Streptococcus uberis* and treated with the gramicidin-mineral oil mixture only one responded satisfactorily. R.P.R.

FEEDS AND FEEDING

66. The Protein Requirements for Milk Production. S. Bartlett, A. S. Foot, S. L. Huthnance and J. Mackintosh, Nat. Inst. for Res. in Dairying, Univ. of Reading, England. J. Dairy Res., *11:* 121–135. 1940.

In a large-scale experiment, using about 500 cows, half the cows were fed a production ration consisting of decorticated groundnut cake, maize meal, wheat offals and molasses supplying about 0.6 lb. protein equivalent per 10 lb. milk produced, and the remaining half were fed a production ration containing the same constituent foods in different proportions supplying a similar starch equivalent, but only two-thirds the amount of protein. No measurable difference was observed in the fatness, handling properties of the skin, hair on the body and percentage of fat and solids-not-fat in the milk.

The milk yields showed no difference during the winter feeding period of about 20 weeks, but during the succeeding period when all the cows were allowed ample grass the milk yield response of the cows previously given the low-protein ration was significantly greater than that of the corresponding cows previously given the high-protein ration.

The experiment was repeated the following winter, using about 600 cows, and confirmatory results were obtained.

Another experiment similarly planned, using 300 cows, compared high- with low-protein production rations consisting of the constituents noted above together with soya-bean meal and rice bran. No significant differences were observed between the effect of the two rations on milk yield or composition, fatness of the cows, etc. There was, however, an indication (not statistically significant) that the low-protein ration which contained a relatively high proportion of rice bran depressed the milkfat percentage.

A small but more completely controlled experiment confirmed the findings of the large-scale experiments. S.T.C.

67. Formation of the Anti Egg-White-Injury Factor (Biotin) in the Rumen of the Cow. L. W. McElroy and T. H. Jukes, Div. of Animal Husbandry and Poultry Husbandry, Univ. of California. Proc. Soc. Exp. Biol. and Med., *45:* 296. 1940.

Experiments were conducted which demonstrated that the anti egg-white-injury (biotin) was formed in the rumen of a cow receiving a ration which was low in this factor. Assuming that the anti egg-white-injury factor is identical with biotin, it appeared that the dried cow rumen contents were a slightly better source of biotin than cane molasses. R.P.R.

68. A Quantitative Study of Vitamins in the Rumen Contents of Sheep and Cows Fed Vitamin-Low Diets. I. Riboflavin and Vitamin K. L. W. McElroy and H. Goss, Div. of Animal Husbandry, Univ. of California, Davis. J. Nutrition, *20:* 527. 1940.

Four sheep and a rumen fistula cow were fed a ration containing less than 0.3 micrograms of riboflavin per gm. The rumen contents of the sheep contained 33 micrograms and that of the rumen fistula cow 25 micrograms of riboflavin per gram. Further evidence of riboflavin synthesis in the rumen was presented by the riboflavin content of milk produced which contained 16 to 18 mg. per day as compared with a maximum intake of 1.8 mg. per day in the feed.

Although the feed of the cow was practically free of vitamin K, the rumen contents were a good source of it. The authors concluded that vitamin K is not a dietary essential in the ration of the cow. C.F.H.

69. A Quantitative Study of Vitamins in the Rumen Contents of Sheep and Cows Fed Vitamin-Low Diets. II. Vitamin B_6 (Pyridoxine). L. W. McElroy and H. Goss, Div. of Animal Husbandry, Univ. of California, Davis. J. Nutrition, *20:* 541. 1940.

The milk of a cow fed a ration deficient in vitamin B_6 contained a normal amount of this vitamin which is evidence of the synthesis of this factor. The ration contained between 1 and 1.5 micrograms of vitamin B_6 while the dried rumen contents contained 8 mg. per gram. This was presented as evidence of the synthesis of this vitamin in the rumen. C.F.H.

70. The Distribution of the "Grass Juice Factor" in Plant and Animal Materials. S. B. Randle, H. A. Sober, and G. O. Kohler, Dept. of Biochem., Univ. of Wisconsin, Madison. J. Nutrition, *20:* 459. 1940.

The distribution of the "Grass Juice Factor" in some of the common food stuffs was studied. Guinea pigs were used as test animals. Dehydrated cereal grass, rye grass, young white clover, peas, pea shells, cabbage, turnip tops and spinach were good sources of this factor, while apples, celery, molasses, peanuts, turnips, lettuce and oats were poor sources.

The young rapidly metabolizing plant tissues are much richer in this growth promoting substance than the older more mature plant material. Much of the original "Grass Juice Factor" can be retained by careful drying and storage at low temperatures. C.F.H.

FOOD VALUE OF DAIRY PRODUCTS

71. Nutritional Facts Worth Promoting by the Milk Dealer. Helen Hunscher, Dept. of Home Economics, Western Reserve Univ., Cleveland, O. Milk Dealer, *30:* 1, 172–182. 1940.

A discussion of adequate diets for human needs is given. Several tables

are presented which show adequate diets and the mineral and vitamin content of different foods. Throughout the article the importance of milk in the diet is stressed. In summarizing, the author states: "We must be conscious of what is in our common foods so that we can aid people in selecting an adequate dietary without increasing the cost out of reason, and so that one does not become fanatic in trying to find this, that or the other constituent. We must teach that we need milk in its various forms, whole grain cereals, green leafy vegetables, a serving of meat, potato and tomato, citrus fruits according to a simple dietary plan given by descriptions of food groups so that everyone can understand and utilize our material." C.J.B.

72. Homogenized Milk for Infant Feeding. Dr. Irving J. Wolman, Children's Hospital, Phila., Pa. Milk Dealer, *30:* 1, 58–60. 1940.

A brief report is given of feeding experiments in which infants were fed the following pasteurized milks: 1. Formulas made from pasteurized unhomogenized milk which was subjected to boiling for 5 minutes in the home; 2. Formulas made from milk homogenized by the sound wave or sonic process; 3. Formulas from milk which had passed through a homogenizer of the low-pressure (750 pounds) type; 4. Formulas from milk which had been forced through a homogenizer of the high-pressure type (2,500 pounds).

The pasteurized homogenized milks used in this study were found to be as good a food for infants as pasteurized milk boiled for five minutes in the home. The high-pressure type homogenizer gave greater reductions in the curd tension of the milk than was obtained by using the other types of homogenizers. In this work, however, milks having curd tension values up to 25 or 30 gms. (break through) gave no difficulties in infant feeding.

C.J.B.

73. Fresh Milk in Infant and Child Feeding. Dr. Henry G. Poncher, Univ. of Ill. Milk Dealer, *29:* 12, 102–105. 1940.

A brief review of the methods of modifying cows' milk for infant feeding. This is followed by a discussion of the various processes used at the dairy for improving the digestibility of milk. The latter methods include homogenization, base exchange, and enzyme treatment. The author states "I do not believe that the soft curd milks are necessarily the solution to our problems in the feeding of infants. Ordinary fresh cow's milk has been successfully modified in the past for infant feeding and this success should continue in the future." C.J.B.

74. The Vitamin B_1 and Riboflavin of Milk. Part I. The Application of Jansen's Thiochrome Test to the Estimation of Vitamin B_1 in Milk. J. Houston, S. K. Kon, and S. Y. Thompson. **Part II. The Different Forms of Vitamin B_1 in Milk.** J. Houston, S. K.

KON, AND S. Y. THOMPSON. **Part III. Effect of Stage of Lactation and of Season on the Vitamin B_1 and Riboflavin Content of Milk.** J. HOUSTON, S. K. KON, AND S. Y. THOMPSON. **Part IV. Comparison of Chemical and Biological Methods of Estimation of Vitamin B_1.** K. M. HENRY, J. HOUSTON, S. K. KON, AND P. WHITE. The National Institute for Research in Dairying, Univ. of Reading, England. J. Dairy Res., *11:* 145–183. 1940.

Part I. Milk was analyzed for vitamin B_1 as follows: A series of three sets of glass-stoppered bottles was set up in duplicate, each containing 6 ml. of milk; 1 ml. of methanol was added to each and 0.20 and 0.25 ml. of a 1 per cent solution of $K_3Fe(CN)_6$ was added to the first and second flask respectively, the third sample being kept as a "blank." Three ml. of a 40 per cent solution of NaOH was added to each and after 1 minute, 13 ml. of redistilled isobutanol. The fluorescence of 10 ml. of the isobutanol layer was measured in a Cohen fluorimeter. A quinine sulphate solution calibrated against crystalline vitamin B_1 was used as a standard. Incubation of the milk with takaphosphatase (240 mg. Parke, Davis and Co. takadiastase per 100 ml. milk) for 3 to 6 hours at 37° C. after adjusting the pH of the milk to 4.0 with normal HCl was found to approximately double the results.

Part II. It was shown by ultra-filtration experiments, by treatment with pepsin and takaphosphatase, with trichloracetic acid and with fuller's earth that vitamin B_1 is present in milk unesterified and phosphorylated and that both forms may be freely diffusible or combined with protein either loosely or by firmer bonds.

Part III. The amount and partition of vitamin B_1 in the milk from six Shorthorn and one Guernsey cow were studied from parturition throughout lactation. Samples of colostrum and early milk were found to contain large amounts of cocarboxylase (up to 80 per cent of the total). Hardly any cocarboxylase was present in mid or late lactation milks. The total vitamin B_1 content was 60–100 μg./100 ml. for colostrum, up to 60 μg./100 ml. for early milk and 30–40 μg./100 ml. for mid and late lactation.

A marked negative correlation was found to exist between the cocarboxylase content of cow's and goat's milk and their alkaline phosphomonoesterase titre. Since this enzyme can not dephosphorylate cocarboxylase it is suggested as probable that it is accompanied in milk by other enzymes more suited to this process.

Feeding and season were found to have little or no effect on the vitamin B_1 content of milk. The riboflavin content of cow's milk, however, was found to be increased by summer feeding. The mean values were 110 μg./100 ml. in winter and 150 μg./100 ml. in summer. The riboflavin content was 3–4 times higher in colostrum than in later milk.

Part IV. The fluorimetric method of assay of vitamin B_1 was compared with the biological test using raw, sterilized, spray and roller-dried and evaporated milks.

When the fluorimetric test measured the total vitamin B_1 and the vitamin B_2 complex in the biological assay was supplied as autoclaved liver the agreement, especially at lower levels of milk feeding, was satisfactory for all but the evaporated milk. The apparent potency of all milks as measured biologically was higher when milk was fed at a high level than when it was fed at a low level. S.T.C.

75. The Influence of Lactose on Calcium Retention in Children. R. MILLS, H. BREITER, E. KEMPSTER, B. MCKEY, M. PICKENS, AND J. OUTHOUSE, Dept. of Home Economics, Univ. of Illinois, Urbana. J. Nutrition, *20:* 467. 1940.

The addition of 36 gm. of lactose to the diet of five boys ranging in age from 5 to 7 years increased calcium retention in every case. The basal diet contained ample calcium and vitamin D. Coincident with the ingestion of lactose there was a statistically significant trend toward a decrease in the calcium lost by way of the kidney. C.F.H.

76. The Determination of Ascorbic Acid in Evaporated Milk, Powdered Milk and Powdered Milk Products. W. W. WOESSNER, C. A. ELVEHJEM AND H. SCHUETTE, Depts. of Chemistry and Biochemistry, Univ. of Wisconsin, Madison. J. Nutrition, *20:* 327. 1940.

Improvements of the method previously suggested by the authors for the determination of dehydroascorbic acid were presented. The analyses of 41 evaporated milks and 9 powdered milks were given. The ascorbic acid content of powdered milk is about equal to that of evaporated milk. The data indicate that neither the spray process, the roller process, nor even the mode of storage, is the significant factor which determines the ascorbic acid content of the powder. The results also indicate that the metallic nature of the manufacturing equipment is not the limiting factor which determines the ascorbic acid content of evaporated milk. The ascorbic acid content of evaporated milk is not affected by the omission or inclusion of the preheating step in the process of manufacture neither by the quality nor quantity of the sterilizing salts. Moreover, the ascorbic acid content cannot be correlated with geographical site of production of the evaporated milk. C.F.H.

77. A Comparison of the Nutritive Values of Raw Pasteurized and Evaporated Milks for the Dog. H. D. ANDERSON, C. A. ELVEHJEM, AND J. E. GONCE, JR., Depts. of Biochem. and Pediatrics, Univ. of Wisconsin, Madison, Wis. J. Nutrition, *20:* 433. 1940.

Whole milk supplemented with iron, copper, manganese and cod liver oil, allowed growth and reproduction (two gestations) in fox terrier dogs. When evaporated milk was fed under similar conditions, most of the young

showed either muscle dystrophy or a hemorrhagic condition of the pericardium, lungs and brain at birth. Vitamin E was effective in curing or preventing the muscle dystrophy but did not prevent the hemorrhagic condition. The young born to the females on fortified pasteurized milk showed some signs of dystrophy and hemorrhage but the symptoms were not as severe as those observed in the case of evaporated milk. All milks supported good growth. C.F.H.

78. Biennial Reviews of the Progress of Dairy Science. Section D. The Nutritional Value of Milk and Milk Products. J. Dairy Res., *11:* 196–224. 1940.

The review covers the two years from the beginning of 1938 to the beginning of 1940. 411 references. S.T.C.

MILK

79. Getting the Sales You Want. Don Francisco, Lord and Thomas Advertising Agency, New York City. Milk Dealer, *30:* 1, 150–162. 1940.

Other industries, especially the California citrus industry, are used as an example of what can be accomplished by telling the people why and how their products should be consumed. The author closes with the following statements:

"Advertising's greatest service to the dairy industry as a whole must eventually come through a concerted movement to raise the per capita consumption of dairy products to a level more nearly approximating American standards of living. The public should have the facts about milk and milk products. Some day it will get them. Some day, either through necessity or foresight, the industry will be given the funds to tell the nation this great health story through an effective educational advertising campaign."

C.J.B.

80. Looking Ahead in the Dairy Industry. Merrill O. Maughan, The Dry Milk Industry. Milk Dealer, *30:* 1, 64–66, 98–102. 1940.

In looking ahead the dairy industry should have a definite goal. The goal according to the author is three-fold: 1. Improved health and economy through a greater use of dairy products; 2. Better dairy products at lower prices; 3. Stabilized markets to the mutual advantage of consumer, producers, manufacturers, and distributors.

The author also discusses the following guide posts as a means of reaching the above-mentioned goal: 1: Public relations; 2. The price of our merchandise; 3. High quality; 4. Constant change; 5. The spread between Class 1 milk and other classes; 6. Heavy and unfair restrictions and trade barriers;

7. More research in our industry; 8. Increased use of manufactured dairy products (except butter); 9. Greater per cent of consumer's dollar to go to farmer; 10. Increased competition; 11. Greater efficiency of methods.

C.J.B.

81. Developing the Southern Dairy Industry. A. D. BURKE, Ala. Poly. Inst., Auburn, Ala. Milk Dealer, *30:* 1, 76–84. 1940.

The author shows why the South is adapted to dairying. This is followed by a discussion of the following fundamental and essential factors in the development of a productive dairy industry: 1. Devotion to livestock; 2. The development of pastures; 3. The production of roughage and feed; 4. The use of proven or indexed sires; 5. Selective evaluation of cows; 6. Health protection; 7. The production of quality products. C.J.B.

82. Laboratory Equipment and Control of Our Products. CARLTON J. AUSTIN, Supplee-Wills-Jones Milk Co., Phila., Pa. Milk Dealer, *29:* 12, 96–100. 1940.

The laboratory control of dairy products is grouped into three broad classifications: *i.e.*, bacteriological, chemical, and physical control methods. The various tests necessary for control work under this classification are listed. The tests are then discussed as they apply to the following sequences of control: 1. Control of raw milk supply on the farm; 2. Control of cooling and transportation of dairy products; 3. Control of pasteurizing process; 4. Control of finished products. C.J.B.

83. Vitamin D Milk. ANONYMOUS. Milk Dealer, *29:* 12, 38, 60–62. 1940.

A survey conducted by the Milk Dealer revealed that it is the larger dealers of the country that are handling vitamin D milk. Of the dealers handling vitamin D milk, 13 per cent of their total output was vitamin D milk. They averaged a daily distribution of 563 quarts of vitamin D milk. Replies from 171 dealers showed that the daily sales of homogenized milk were 717 quarts per dealer, and 101 dealers handling homogenized vitamin D milk averaged daily sales of 675 quarts. The results of the survey are also given according to sections of the country. C.J.B.

84. Your Quality Improvement Program. JOSEPH BURNS, Schwartz Mfg. Co., Two Rivers, Wis. Milk Dealer, *29:* 12, 36, 59. 1940.

Educating the dairy farmer in the science of producing high-quality milk is essential in a quality improvement program. The author emphasizes the necessity of filtering milk on the farm. C.J.B.

85. Papaya. HARPER MOULTON, Univ. of Mo. Milk Dealer, *29:* 12, 32–33, 72. 1940.

A history and description is given of papaya. This is followed by a dis-

cussion of its use in the dairy industry. Papaya is a tropical fruit resembling a melon. It is reputed to be high in vitamin A, B, C and D. The author states, "In a new drink called 'papaya milk' we find something of vital interest to milk dealers which promises a greatly increased consumption of milk." The author believes that this milk drink will stop the inroad the soft beverage industry is making upon milk consumption. C.J.B.

PHYSIOLOGY

86. The Restoration of Ovulatory Cycles and Corpus Luteum Formation in Persistent-Estrous Rats by Progesterone. JOHN W. EVERETT, Dept. of Anatomy, Duke Univ. Endocrinology, *27:* 681. 1940.

Regular ovulatory cycles were restored in 23 out of 24 persistent-estrous rats by the daily administration of small sub-inhibitory doses of progesterone. The effective daily doses ranged from 0.25 mg. to 1.0 mg. of progesterone. Ovulation and corpus luteum formation were induced in rats whose history indicated little likelihood of such events occurring naturally. The initial effect of progesterone treatment was the appearance of diestrus, usually without luteinization, within 48 hours. Diestrus was of ordinary duration in each instance and a cycle followed whenever a sufficient amount of progesterone was injected at the end of diestrus. Otherwise, persistent estrus recurred promptly. R.P.R.

87. Induction of Sexual Receptivity in Estrogen Conditioned Spayed Female Guinea Pigs by Orally Administered Progesterone and Pregneninolone. ARNOLD L. SODERWALL, Labs. of Primate Biology, Yale Univ. Endocrinology, *27:* 840. 1940.

One mg. of orally administered progesterone was sufficient to induce normal copulatory behavior in a high percentage of guinea pigs previously conditioned with subcutaneously injected estrogen. Lower doses were also effective; however, the percentage of animals responding was smaller. Two mg. of pregneninolone was effective in 8 of 8 animals and one mg. was effective in 10 of 16 animals. R.P.R.

MISCELLANEOUS

88. Operation, Maintenance and Inspection of Boilers. J. F. HUNT, Hartford Steam Boiler Inspection and Ins. Co., New York City. Nat. Butter and Cheese J., *31:* 11, 56. 1940.

Careful operation and a well managed inspection routine can keep accidents to power equipment to a minimum. The installation of boilers should meet the specifications of the Boiler Construction Code of the American Society of Mechanical Engineers. Safety appliances should be properly installed and kept in good operating condition. Corrosion, a serious safety

problem, may be of the internal or external type. Internal corrosion may be pitting, grooving or general internal corrosion. Pitting, caused by entrained oxygen, can be eliminated by deaerating processes; grooving is a localized corrosion found on internal surfaces of sharp flanges or along joints; general internal corrosion attacks the metal uniformly and can be prevented by proper water treatment. External corrosion occurs where moisture comes in contact with exposed surfaces and air. Sooty or ash covered heating surfaces of unused boilers deteriorates rapidly. External corrosion will take place on heating surfaces moistened from a leaking tube, handhole, gasket or soot blower and is especially apt to occur with the heads of the lower drums of water tube boilers. "Caustic embrittlement" usually evidences itself by persistent leakage and breaking off of rivet heads; the condition, if not too bad, can be remedied by placing wider joint straps and by feed water treatment to give the sulfate-carbonate alkalinity ratio of the A.S.M.E. Code. Combustible fuel suspended in air in appreciable quantities forms an explosive mixture which makes extreme care necessary in plants equipped for using gas, oil or pulverized coal fuels. Superheaters may be a source of unnecessary maintenance cost; adequate circulation of steam is vital to the life and safety of this equipment. W.V.P.

89. What to do About Floors. L. C. THOMSEN, Univ. of Wis., Madison, Wis. Nat. Butter and Cheese J., *31:* 11, 17. 1940.

Dairy plant floors should be free of hollows, impervious to water, washing or soaker solutions, milk wastes, fats, etc.; they should be easy to clean, easy to work on and have strength to stand loads and traffic. Concrete floors have many of these properties but brick is coming into more common use. The laid concrete floor will have a base reinforced with rods. The wearing surface will be at least one inch thick, will consist of a one-one-two (cement-sand-coarse aggregate) mixture and three and a half to four and three-quarters gallons of water for each sack of cement, and be carefully tamped and troweled. Surface treatments to protect concrete against the acids of dairy wastes are available. Water, steam or other pipes laid in the floor tend to cause cracks. Brick floors resist acids and although they are expensive to install they are probably cheapest in the long run. All floors should slope one eighth to one quarter inch per foot to adequate drains. W.V.P.

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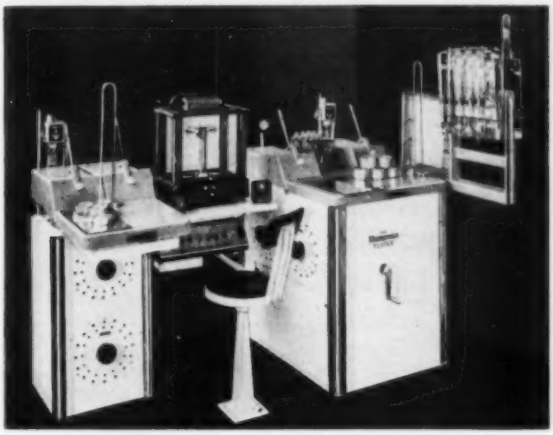

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Membership. Any person is eligible to membership who is formally announced by an Agricultural College or Experiment Station, or by the Bureau of Dairy Industry of the United States Department of Agriculture or by the Canadian Department of Agriculture as an instructor, extension worker, investigator, or administrative officer connected with the dairy industry, or any person filling a position of responsibility connected with the dairy industry who has had a college or university training in technical science, or any person filling a responsible position in the dairy industry of a professional character requiring a technical knowledge of dairying of a high order. The membership fee is $5.00.

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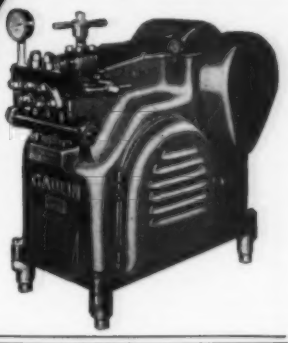

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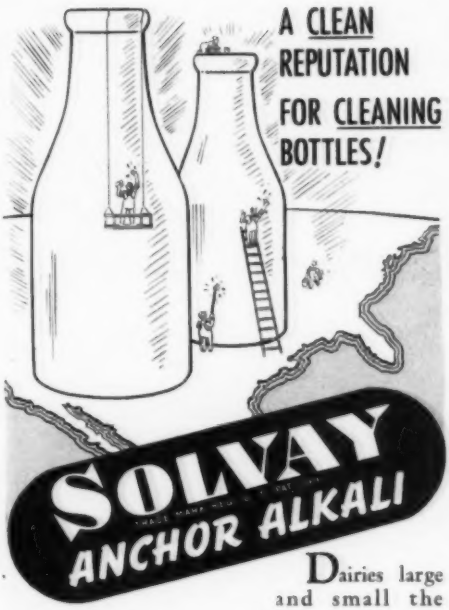

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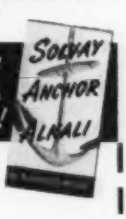

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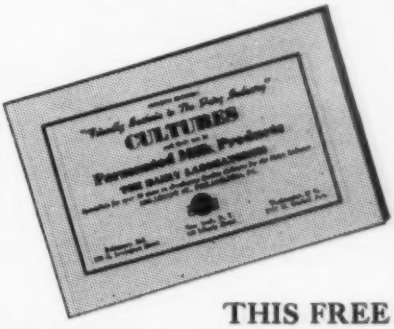

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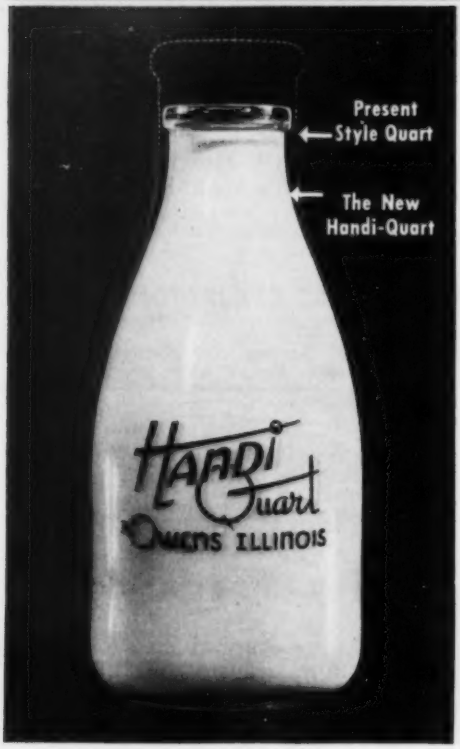

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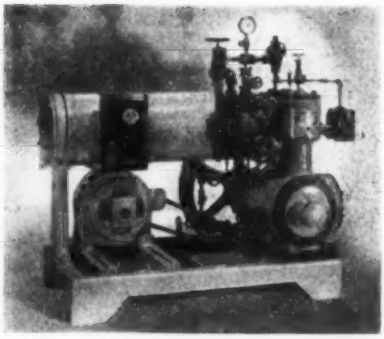

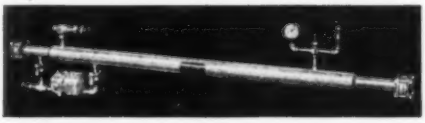

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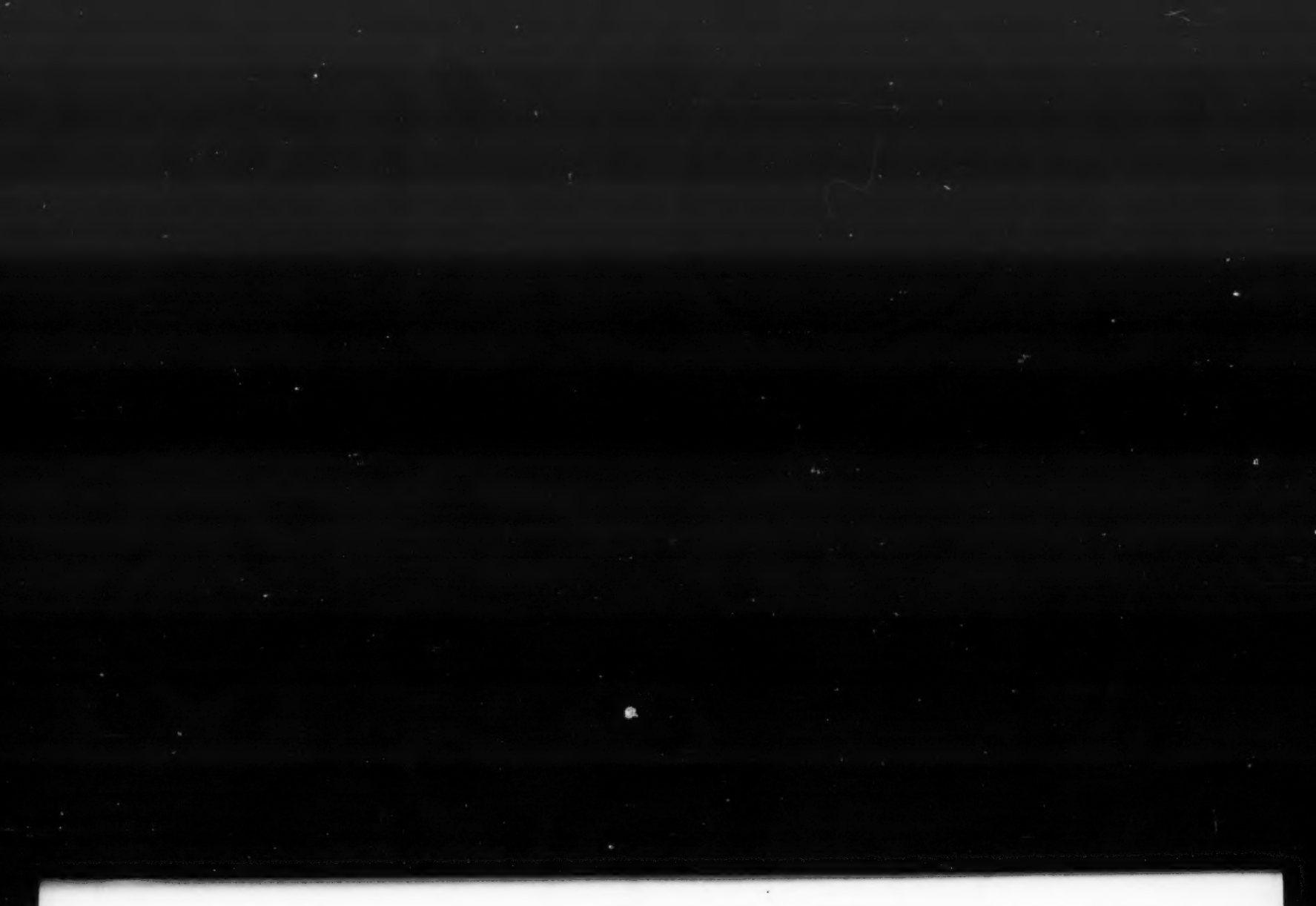
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